Spatial and Temporal Heterogeneity of Malaria Transmission in sub-Saharan Africa

Thesis

Spatial and Temporal Heterogeneity of Malaria Transmission in sub-Saharan Africa

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Statement of Originality

I hereby certify that the contents of this thesis are to the best of my knowledge, my own research work. This thesis has not been submitted for any other purpose elsewhere. Any assistance provided during thesis writeup and analysis has been duly acknowledged.

Sign _____  _____

Polycarp Kambona Mogeni

Abstract

The past decade has seen a marked decline in malaria transmission and substantial progress towards malaria control in parts of Africa. A key tool in malaria control is the insecticide treated net (ITN). However, malaria remains a public health emergency in sub-Saharan Africa with children under five years of age bearing the greatest burden of the disease. In this thesis, I examine three sets of questions that are relevant to the changing epidemiology of malaria.

a) I investigated trends in malaria cases in Kilifi County on the Kenyan Coast over geographical area and over time, age, and the effectiveness of ITN use in the community. I observed a decline in the proportion of admitted children who had malaria parasites detected by microscopy from 1998 to 2009 as previously observed in some countries of sub-Saharan African. However, there was a steady and marked increase in the proportion of children with malaria parasites after 2009 accompanied by a shift in burden of disease from younger age groups to older age groups. Community ITN use was highly effective in reducing the risk of malaria. As transmission fell, geographical heterogeneity became more marked.

b) I undertook an analysis of data shared from 19 studies conducted between 1996 and 2015 in 7 countries of sub-Saharan Africa to examine whether micro-geographical heterogeneity was generalizable. Hotspots were identified in all datasets, and were more marked at lower transmission intensity. Given the predictability with which hotspots occur as transmission intensity falls, malaria control programmes should have a low threshold for responding to apparent clustering of cases.

c) I then considered whether rapid diagnostic tests (RDTs) or PCR would alter detection of hotspots. Using cross-sectional studies of asymptomatic parasitaemia, I describe hotspots of malaria transmission in three sites on the Kenyan Coast using data from 8581 study participants. Microscopy and RDT missed a larger proportion of infections in low transmission settings. PCR hotspots completely overlapped with microscopy hotspots at a moderate transmission, but not at two low transmission setting. From this work, I recommend that malaria control programmes consider PCR testing for targeted control when transmission intensity is low.

Candidate's Contribution

This work utilized a longitudinal pediatric admission surveillance data for febrile malaria collected at the Kilifi County hospital for a 25-year period, data assembled from multiple sites in sub-Saharan Africa and yearly cross-sectional surveys for asymptomatic parasitaemia conducted between 2007-2016 in three sites within Kilifi county. My primary responsibilities were as follows:

- In the analysis of febrile malaria using Kilifi County Hospital data; I was responsible for the design and implementation of the analysis plan, cleaning clinical and ITN use data and quality assurance, remote sensing data extraction, statistical analysis, manuscript writing and submission for publication.
- In the multicentre analysis work; I was responsible for the design and implementation of the analysis plan, data cleaning and quality assurance, communication with the principal investigators from various centres during data cleaning, statistical analysis, manuscript writing and submission for publication.
- In the study of asymptomatic parasitaemia in the three sites within Kilifi county; I was responsible for the design and implementation of the analysis plan, data cleaning and quality assurance, statistical analysis, manuscript writing and submission for publication.

Acknowledgements

I am deeply indebted to my supervisors; Professor Philip Bejon and Professor Thomas Williams of the KEMRI-Wellcome Trust Research Programme in Kenya and Professor Gregory Fegan of the Swansea University Medical School for their tireless efforts in guiding me through the wide field of malaria epidemiology. I am grateful to my director of studies Prof. Philip Bejon for welcoming me to his research group in Kilifi and for his patience, constant stimulating discussions and support. I owe gratitude to my 3rd party monitor Prof. James Berkley and my mentor Prof. Olubayi Olubayi for constant encouragement.

The multicenter study would not have been possible without the generous data contribution from principal investigators of the various centers from sub-Saharan Africa. I am indebted to the Hotspots Group Authors for this generous gesture. Similarly, the febrile malaria study at the Kilifi county hospital and the analysis of asymptomatic parasitaemia would not have been possible without the dedication of the KEMRI-Wellcome Trust laboratory staff. I thank Brett Lowe, Ken Awuondo, Gabriel Mwambingu, Joyce Ngoi, Domtila Kimani, Richard Morter among others for steering the laboratory aspect of these work. I thank the Kilifi county hospital clinical staff and fieldworkers who participated in clinical data collection.

I am appreciative to my colleagues Irene Omedo, Alice Kamau, David Kangoye and Kevin Wamae for the insightful discussions we shared over the years. I thank KEMRI-Wellcome Trust data managers for their timely response to my many data requests. Particularly Ken Mwai and George Nyangweso for the assistance rendered even within short notices. I thank Alex Maina for the library services.

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I am profoundly grateful to my family for believing in me and always supporting and encouraging me through the study and writeup period.

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The work in **Chapter 3** has been published in **PloS Medicine** as:

Mogeni P, Williams TN, Fegan G, Nyundo C, Bauni E, et al. (2016) Age, Spatial, and Temporal Variations in Hospital Admissions with Malaria in Kilifi County, Kenya: A 25-Year Longitudinal Observational Study. PLoS Med 13(6):

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The work in **chapter 5** has been published in the **Journal of Infectious Disease** as:

Mogeni P, Williams TN, Omedo I, Kimani D, Ngoi JM, et al. (2017) Detecting malaria hotspots: a comparison of RDT, microscopy and polymerase chain reaction. JID.

Chapter One

1 Literature review

1.1 The malaria parasite

Malaria is a highly infectious disease caused by parasites of the genus *Plasmodium* and is transmitted to humans through bites from infected female mosquitoes of the genus *Anopheles*. Six parasitic species of *Plasmodium* cause human malaria; *P. falciparum, P. vivax, P. malariae, P. ovale* (split into two species: *P.o. curtisi and P.o. wallikeri), (Carter and Mendis 2002, Singh et al. 2004, Sutherland et al. 2010)* and *P. knowlesi (Cox-Singh et al. 2008, White 2008, Sabbatani et al. 2010).*

P. falciparum and *P. vivax* are the most commonly researched parasite species because of their wide geographical coverage, high morbidity and mortality. *P. falciparum* is highly pathogenic, associated with the highest number of deaths and accounts for >95% of malaria infections in sub-Saharan Africa. This malaria parasite is characterized by cyclic patterns of fever and chills that in some instances leads to severe forms of malaria (i.e. severe malaria anaemia common in children and cerebral malaria common in older children) that are associated with high case fatality. Outside Africa, *P. vivax* accounts for the highest burden of malaria (>50% of all malaria *(Mendis et al. 2001)*), but it is less common (<10% of all malaria cases) in sub-Saharan Africa partly due to the absence of the Duffy bood-group-antigen *(Howes et al. 2011, Gething et al. 2012)* that the parasite requires to invade red blood cells *(Miller et al. 1976).* However, emerging evidence show that *P. vivax* can infect and cause disease in some Duffy negative individual *(Menard et al. 2010, Mendes et al. 2011)* suggesting a possible interaction with other biological processes or a possibility of other receptors aiding cell invasion.

Infections with *P. vivax* are characterized by relapses arising from persistent liver stages of the parasite, and lower parasite densities compared to *P. falciparum*. Whilst *P. ovale* and *P. malariae* have historically been perceived to be less prevalent, recent reports show that they could be more prevalent in sub-Saharan Africa than previously predicted and are often undetected by light microscopy *(Doderer-Lang et al. 2014)*. Nevertheless, disease due to these species are relatively mild and easily treated by commonly used anti-malaria drugs *(Mueller et al. 2007)*. In terms of survival, *P. ovale* and *P. vivax* are best adapted to long-term survival in the human host due to their latent erythrocytic forms. Recent evidence shows that *P. falciparum (Ashley and White 2014)* and *P. malariae (Collins and Jeffery 2007)* can also persist asymptomatically for decades.

P. knowlesi is mainly found among South-Asian primates (long tailed and pig-tailed macaques), and infects humans more frequently than previously thought. Morphologically, *P. knowlesi* resembles *P. malariae* making it difficult to distinguish by microscopy, and is potentially life-threatening given its short blood stage cycle (24 hour cycles) accompanied by high parasitaemia and fever *(Singh et al. 2004, Cox-Singh et al. 2008)*. Research into the epidemiology of *P. knowlesi* malaria increased recently when it was observed that the species could infect humans. Human infection with *P. knowlesi* appears to have resulted from continued human encroachment into non-human primate habitats, who are the natural hosts. *P. knowlesi* is likely to pose a challenge during elimination stages of malaria as humans continue to encroach on primate habitats in South East Asia.

However, *P. knowlesi* transmission has not been reported in Africa, and this may partly be associated with the absence of the reservoir host (long tailed and pig-tailed macaques) *(Moyes et al. 2014)*, and the high prevalence of the Duffy negative genotypes among the African

population *(Miller et al. 1975)*. Although there is a possibility that human to human transmission may occur in areas with high density *P. knowlesi* zoonosis, there is currently no evidence to firmly establish that such transmission takes place *(Ramasamy 2014)*.

This thesis hereafter focuses on the epidemiology of *P. falciparum* in sub-Saharan Africa except chapter five that examines the prevalence and geographical distribution of other malaria species on the Kenyan coast.

1.2 Life cycle

P. falciparum, the most lethal form of malaria, has a sophisticated life cycle making it difficult to control. The parasite undergoes several developmental stages in both the mosquito and the human hosts (Figure 1.1).

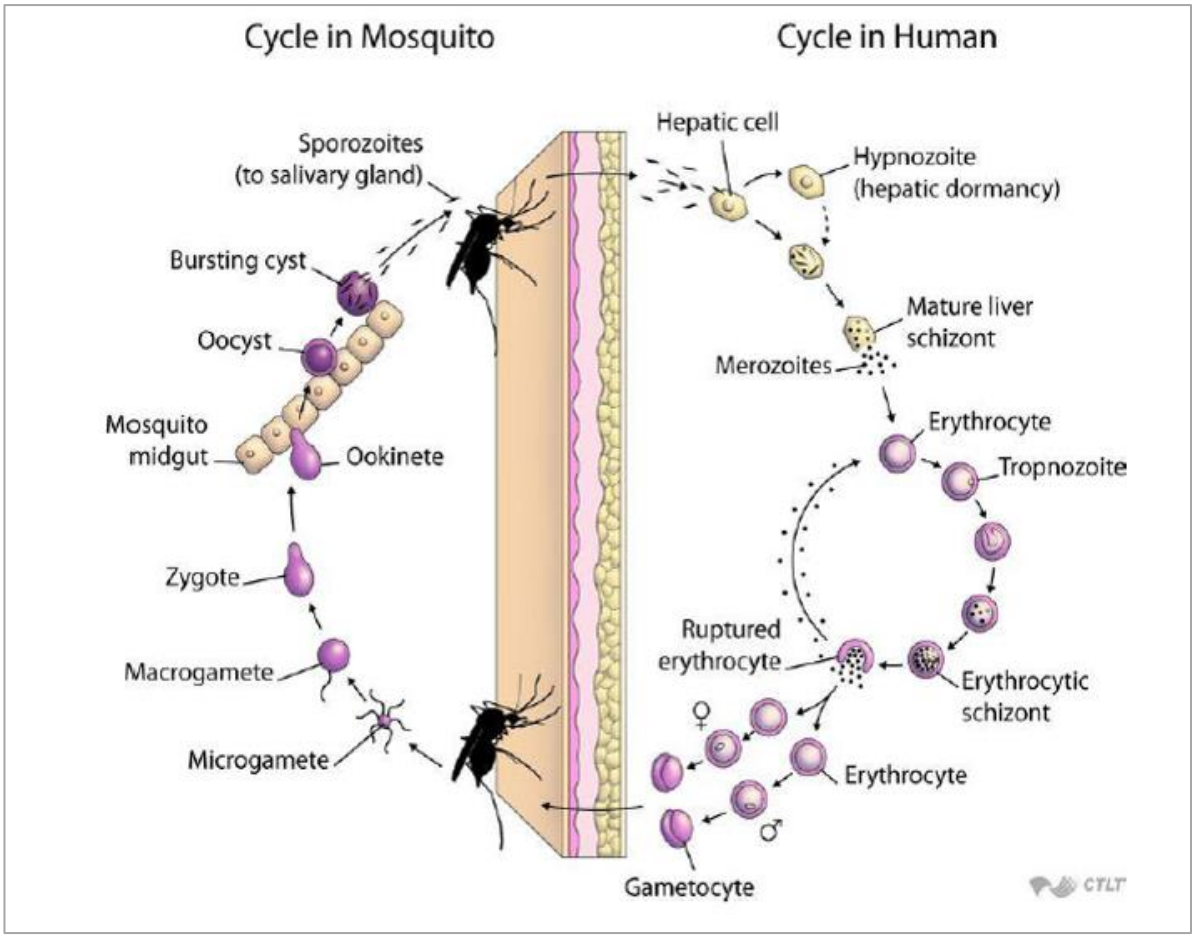

Figure 1.1: ***P. falciparum malaria lifecycle showing the asexual phase in the human host and the sexual stage in the female Anopheles mosquitoes.***

(Wirth 2002)

The cycle begins when a female infected *Anopheline* mosquito injects sporozoites into the human host when feeding from a blood meal. The sporozoites are then transported to the liver via the bloodstream and invade the hepatocytes. Within the hepatocyte, the sporozoites undergo proliferation and differentiation culminating in the liberation of merozoites. Several rounds of asexual multiplication give rise to tens of thousands of haploid merozoites over a period of 6–16 days. Merozoites are then released into the bloodstream and invade erythrocytes. Once the erythrocytes have been invaded, merozoites undergo a process of growth and asexual multiplication to produce daughter merozoites over a period of 48 hours through ring stages

(trophozoite) to form schizonts which consist of many mature newly formed merozoites. Infected red blood cells (RBC) then lyse and liberate the merozoites which immediately invade new erythrocytes to begin a new erythrocytic cycle. This blood stage infection is responsible for the clinical symptoms of malaria, especially fever. Not all the ring trophozoites mature into schizonts. A small proportion undergo morphological changes and sexual differentiation to become male and female gametocytes. The gametocytes mature after about 10 days and are ready to be taken up by a blood meal seeking mosquito for the vector phase.

Following an infected blood meal, the anopheline mosquito ingests both male and female immature gametes circulating in an infected human's bloodstream. Under suitable environmental conditions (i.e. temperature), the male and female microgametes differentiates into macrogametes and fuse to form a zygote which undergoes a series of differentiation and growth stages that result in the production of infective sporozoites in the salivary glands of the mosquito, thus completing the cycle *(Beier 1998, Garcia et al. 2006).*

1.3 Malaria disease burden

The global burden of malaria has markedly declined over the past 15 years (Figure 1.2) in association with improving access to first-line anti malaria treatment and vector control *(Murray et al. 2012, WHO 2012c, Noor et al. 2014, Bhatt et al. 2015a).* During this period, malaria related mortality declined by ~60% globally. On the global scale, the World Health Organization (WHO) estimates ~262 (95% confidence interval[CI] 205-316) million cases, with ~839,000 (95%CI 653,000 – 1.1 million) malaria related mortality in 2000 *(WHO 2015b)*; and ~214 (95%CI 149-303) million cases, with ~ 438,000 (95%CI 236,000 – 635,000) malaria related mortality in 2015 *(WHO 2015b).*

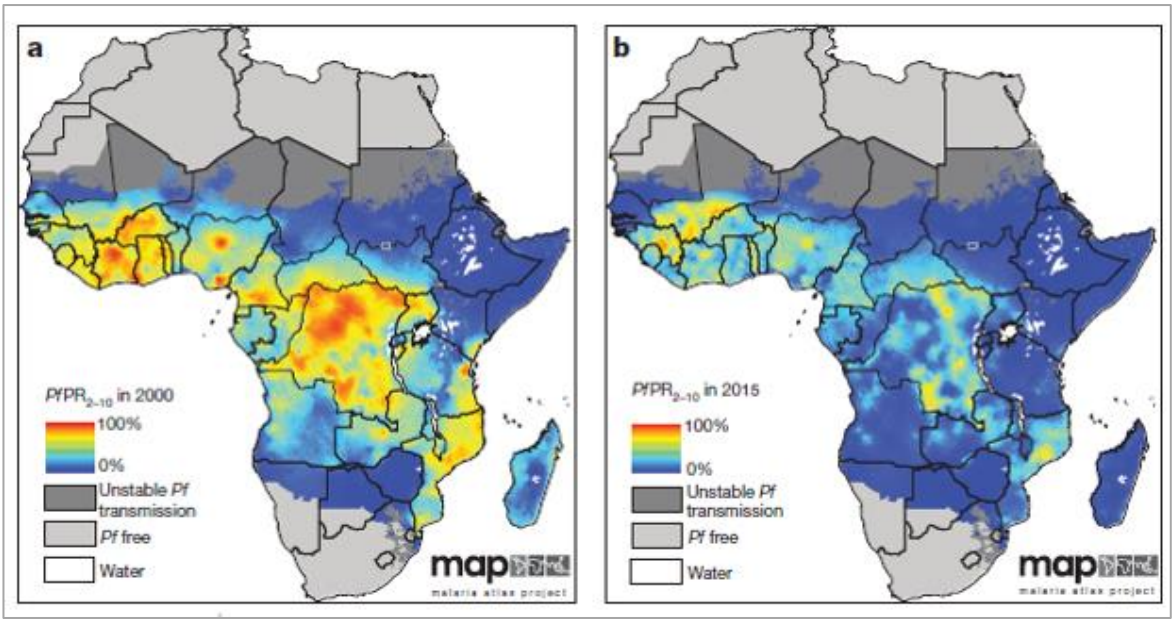

Figure 1.2: ***Changes in infection prevalence in Africa 2000-2015.***

Panel ***a,*** *shows* PfPR_{2-10} *for the year 2000 predicted at 5×5 km resolution and panel* ***b,*** *shows* PfPR_{2-10} *for the year 2015 predicted at 5×5 km resolution (Bhatt et al. 2015a).*

Most deaths occurred in sub-Saharan Africa (90%) followed by South-East Asia (7%) and Eastern Mediterranean region (2%) *(WHO 2015b).* The high burden of malaria in Africa has largely been attributed to the existence of an effective vector species that include; *Anopheles (An.) arabiensis, An. funestus and An. gambiae* (Figure 1.3) *(Sinka et al. 2012). An. gambiae*, the most important vector in Africa, preferentially feeds on humans hence transmitting malaria from an infectious human host to susceptible individuals. This species has a documented long lifespan that consequently increases the likelihood of transmitting the parasite *(Beier 1998)*. The humid, temperate and stable weather conditions in Africa enable the vector to thrive and the parasites to complete its lifecycle. In addition, social and economic factors (poverty) play an important role in sustaining malaria transmission in Africa *(Greenwood and Mutabingwa 2002).*

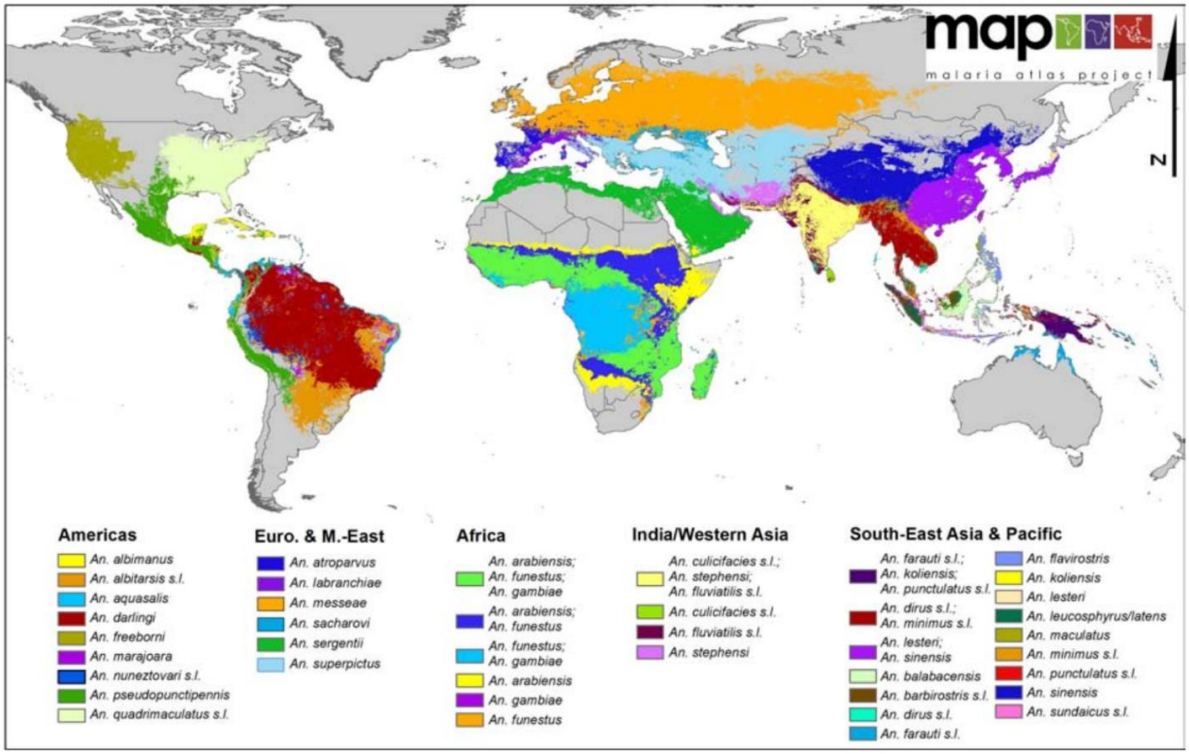

Figure 1.3*: A global map of dominant malaria vector species* *(Sinka et al. 2012).*

Besides the WHO, there are other groups that have characterized malaria transmission intensity, mortality attributable to malaria and effect of malaria control intervention in sub-Saharan Africa *(Gething et al. 2011, Murray et al. 2012, Noor et al. 2014, Bhatt et al. 2015a, Gething et al. 2016)*. Overall, there is a consensus on the marked spatial and temporal heterogeneity of malaria transmission intensity in Africa. However, the magnitude of reported estimates varies considerably requiring careful interpretation *(Nkumama et al. 2017)*. For instance, Bhatt et al predict that nearly all sub-Saharan African counties experienced marked decline in $pfPR_{2-10}$ transmission intensity *(Bhatt et al. 2015a)*. This is consistent with some studies in individual countries (for instance Kenya *(O'Meara et al. 2008, Snow et al. 2015)*, Tanzania *(Mmbando et al. 2010)* and the Gambia *(Ceesay et al. 2008)*) but studies conducted in Burkina Faso and Uganda show little evidence of declining transmission and in some instances, depict an

increasing trend *(Okiro et al. 2011, WHO 2015b)*. The inconsistencies in the global estimates of morbidity and mortality arise from the inherent challenges in the sparsity of data collection and modelling assumptions commonly undertaken. Model-based geostatistical methods rely on borrowing information to estimate areas where data are unavailable *(Noor et al. 2014)*. This concept is anchored on Waldo Tobler's first law of geography that states "Everything is related to everything else, but near things are more related than distant ones"*(Tobler 1970)*. However, heterogeneity of transmission is seen at a range of scales *(Bejon et al. 2014)* and therefore assessing the true burden of malaria in Africa is hampered by a lack of data that results from incomplete or limited medical records on malaria cases, limited laboratory testing given that most malaria cases and deaths occur outside health-care facilities and are thus inaccessible for recording *(Snow et al. 1999, Greenwood and Mutabingwa 2002, WHO 2012c)*.

Health monitoring and evaluation of malaria attributable deaths, within Africa, frequently relies on verbal autopsy (VA) to derive cause of death *(Murray et al. 2012, Gething et al. 2016)*. VA tools and procedures are not standardized, and vary substantially among sites *(Soleman et al. 2006)* and their accuracy is notably lower than medical certification of causes of death *(Murray et al. 2014)*.

In addition to the measurable global morbidity and mortality associated with malaria, there is evidence that malaria is associated with reduced birthweight, premature births, still births, cognitive impairment and social economic effects. Infants born premature or with low birthweight are more susceptible to infectious diseases and are at an increased risk of death compared to term babies with normal birthweight *(Katz et al. 2013, Bousema et al. 2014, Chen et al. 2016, Lawn et al. 2016)*.

1.4 Malaria pathophysiology and immunity

When a susceptible individual is inoculated with malaria parasites, a sequence of manifestations follows as shown in figure 1.4 below. However, progression depends on: 1) anti-disease immunity, 2) host factors, 3) efficacy and timing of antimalaria drugs, and coinfection.

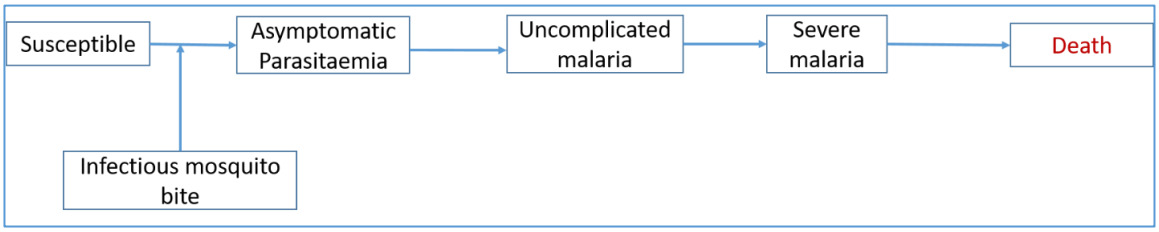

Figure 1.4*: Malaria pathophysiology*

1.4.1 Clinical malaria

The typical clinical symptoms of malaria are cycles of fever and chills and occur among semi-immune or non-immune individuals. In malaria-endemic areas, clinical malaria is a rare feature during infancy *(Murungi et al. 2017)*. This is because passively acquired maternal IgG antibodies are thought to confer protection against malaria during the first few months of life *(Edozien et al. , Murungi et al. 2017)*. However, maternal immunity against malaria is short lived (~6 months) subsequently predisposing children to infection later in life *(Murungi et al. 2017)*.

Severe malaria manifests in three overlapping syndromes: 1) severe malaria anaemia, 2) impaired consciousness and 3) respiratory distress (Figure 1.5) *(Marsh et al. 1995)*.

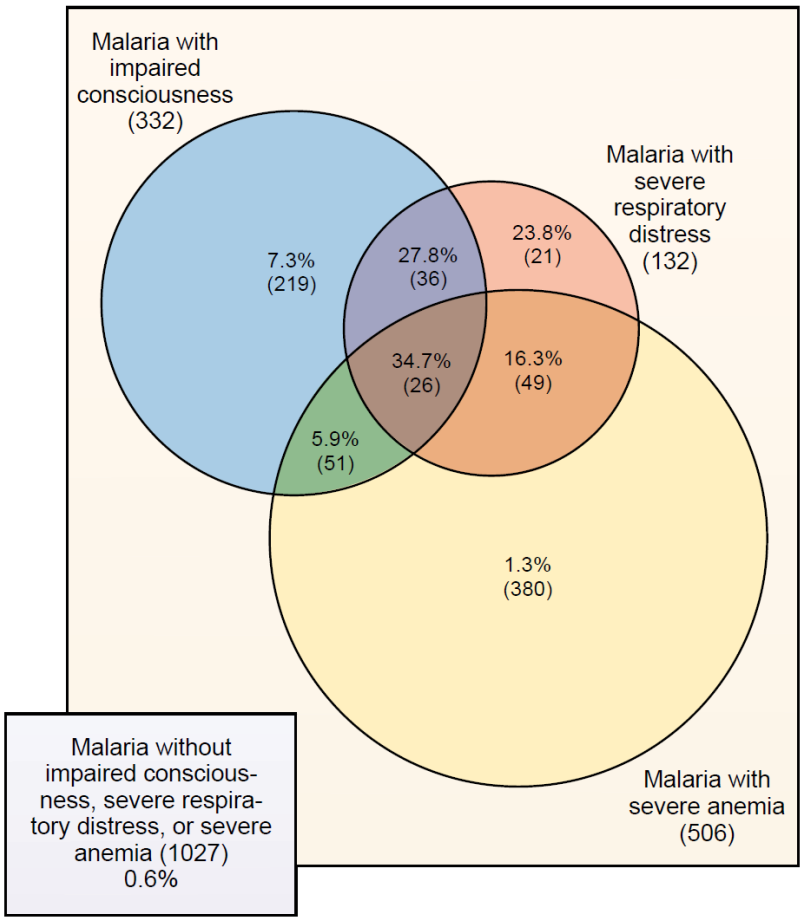

Figure 1.5*: Prevalence, overlap, and mortality for major clinical subgroups of severe malaria.*

Total numbers are given in parentheses, and mortality is given as a percentage (Marsh et al. 1995).

In high transmission areas, severe malaria anaemia is predominant and affects mainly younger children, while in low transmission areas cerebral malaria is more common in older children and is associated with high case fatality *(Snow et al. 1994)*. Children in malaria endemic zones acquire immunity to clinical malaria rapidly following repeated infections, and hence, there is an inverse relationship between transmission intensity and age of susceptibility to clinical malaria *(Snow et al. 1997, Okiro et al. 2009)*. However, anti-parasite immunity develops slowly and is incompletely acquired even among adults living in endemic areas. Some have argued that

immunity may suppress infection but is never sterilizing *(Doolan et al. 2009)*. Furthermore, there is evidence to show that immigrant adults, from endemic areas, living in malaria free regions lose their protective immunity and become susceptible again *(Marsh and Kinyanjui 2006)*. In addition, malaria coinfection with some diseases may alter the development of immunity and enhance development of malarial symptoms. For instance, coinfection with HIV-1 has been associated with increased rate of malarial fevers *(French et al. 2001)*, higher parasitaemia, more complications and a higher case fatality rate *(Hendriksen et al. 2012)*. Similarly, Mendelian randomization studies using Sickle Cell trait as an instrumental variable have shown that malaria increases susceptibility to non-typhoidal salmonella infection *(Scott et al. 2011)*, confirming prior epidemiological observations *(Mabey et al. 1987, Mackenzie et al. 2010)*. The mechanism appears to be related to haemazoin production by the parasite interacting with host immunity *(Cunnington et al. 2012)*.

1.4.2 Asymptomatic parasitaemia

Asymptomatic parasitaemia is defined as the presence of parasitaemia in the absence of fever or other acute symptoms *(Lindblade et al. 2013)*. Continued exposure to malaria parasites in endemic areas often results in the development of anti-disease immunity *(Marsh and Kinyanjui 2006)*. However, this kind of immunity does not protect against re-infection but controls parasitaemia to levels where the infection cannot cause disease. Whilst individuals with clinical malaria develop symptoms and typically seek medication, individuals with asymptomatic parasitaemia do not and hence maintain a reservoir for onward transmission in the community *(Bousema et al. 2014, Chen et al. 2016)*.

1.5 Malaria diagnostic tools

Presumptive diagnosis of febrile cases as malaria was common in sub-Saharan Africa when diagnostic tools were not widely available *(Nankabirwa et al. 2009)*. However, many infectious diseases cause fever leading to a substantial proportion of febrile cases presenting to under-resourced health facilities or retail outlets being treated presumptively for malaria in the absence of diagnostic testing *(Amexo et al. 2004, Nankabirwa et al. 2009)*. In addition to misuse of anti-malarials, over-diagnosis also diverts attention from other causes of severe illness and may lead to adverse clinical outcomes *(Reyburn et al. 2004)*.

WHO recommends that malaria case management be based on parasitological diagnosis *(WHO 2012b)*. Rapid diagnostic tests (RDTs) and microscopy are the primary diagnostic tools for confirmation of suspected clinical malaria in most resource limited settings *(WHO 2013a)*. Clinical malaria is usually accompanied by fever and readily detectable parasitaemia. On the other hand, nucleic acid amplification-based diagnostic tools are recommended for the detection of asymptomatic parasitaemia in areas of low transmission intensity where submicroscopic infections are more common *(Okell et al. 2009, Okell et al. 2012, WHO 2013a)*.

Light microscopy examination of stained blood smears remains the gold standard for laboratory diagnosis of malaria. Although microscopy is relatively inexpensive compared to molecular diagnostics, it is not accessible and affordable in smaller health facilities in Africa. Microscopy can be highly sensitive and specific when conducted by well-trained laboratory personnel in high transmission settings, but suffers from detection limits, particularly in low transmission settings where clinical illness may be associated with lower parasitaemias *(Okell et al. 2012)*. Moreover, there are challenges with quality assurance and standardization across sites or across independent laboratories. The advent of RDTs has greatly expanded diagnostic coverage due to

their simplicity, cost effectiveness and availability when compared to microscopy and molecular based diagnostics. RDTs are currently widely used in endemic areas and have been shown to be highly sensitive and specific compared to light microscopy *(Hendriksen et al. 2011)*. RDTs measure the presence of *P. falciparum* histidine-rich protein 2 (HRP2) and/or lactate dehydrogenase (LDH) *(Moody 2002)* with important limitations. That is, HRP2 antigen can circulate in blood for weeks after treatment leading to false positives, and some parasites do not express the HRP2 protein rendering the tool less sensitive *(Cheng et al. 2014)*. LDH on the other hand, persists less than HRP2 leading to fewer false positives *(Hopkins et al. 2007, Murray et al. 2008, Houze et al. 2009)*. These caveats aside, HRP2 based RDTs have well documented lower detection limits in the range of 100 to 200 parasites per microliter *(WHO 2012b)*. In addition, poor transport and storage of RDTs with sustained exposure to high temperature can potentially affects their diagnostic performance *(Cheng et al. 2014)*.

Recent advances in molecular techniques have led to increased sensitivity in the detection of malaria parasites. A set of PCR techniques for malaria diagnosis are currently available and include single step, nested, multiplex and quantitative PCR. Although these techniques are highly sensitive, they are expensive and requires highly trained personnel to operate, and are thus primarily used for research purposes. Other nucleic acid amplification technology currently in use include the loop-mediated isothermal amplification (LAMP) and nucleic acid sequence-based amplification (NASBA) that do not need thermocyclers to operate *(Erdman and Kain 2008)*. These techniques are faster, relatively cheap and easy to operate compared to PCR but with equal sensitivity and can potentially be used in rural settings outside the research environment.

1.6 Effective case management

Early diagnosis and prompt effective treatment is one of the key strategies for malaria control. Uncomplicated *P. falciparum* malaria is defined as confirmed parasitaemia (by microscopy or RDT) and fever but without signs of severe illness or vital organ dysfunction. WHO recommends a three days regimen of artemisinin-based combination therapies (ACT) for treatment of uncomplicated malaria in both children and adults except pregnant women in their first trimester *(WHO 2015a)*. Both artesunate plus amodiaquine (ASAQ) and artemether-lumefantrine (AL) and Atovaquone-proguanil (AP), have been shown to be efficacious in the treatment of uncomplicated malaria *(Martensson et al. 2005)*. In low transmission settings, efforts to reduce transmissibility of treated *P. falciparum* infections to mosquitoes may be boosted by an additional single dose of primaquine in combination with ACT *(WHO 2015a)*. Although there are concerns regarding resistance in South East Asia *(Ashley et al. 2014),* ACT for treatment of uncomplicated malaria remains highly effective in Africa, but coverage is substantially heterogeneous and remains unacceptably low in some areas. Approximately 80.3% of children with uncomplicated malaria did not have access to ACT in 2015 *(Bennett et al. 2017).*

Adults and children with severe malaria (defined as confirmed parasitaemia by microscopy or RDT with one or more of the following: impaired consciousness, prostration, multiple convulsions, acidosis, jaundice, hypoglycaemia, severe malarial anaemia, renal impairment, among other signs) should be treated with intravenous or intramuscular artesunate for at least 24 hours of parenteral therapy followed by 3 days of ACT once oral therapy can be tolerated *(WHO 2015a)*. Artesunate is superior to quinine in averting mortality due to severe malaria *(Dondorp et al. 2005, Dondorp et al. 2010)*.

1.7 Malaria control activities

1.7.1 Historical malaria control activities.

The history of malaria control is well documented in literature and dates to the 20th century. Early malaria control methods mainly relied on vector control through insecticide residual spraying with dichlorodiphenyltrichloroethane (DDT) and drug administration using chloroquine *(Killeen et al. 2002)*. Laviciding has been credited in controlling malaria in the northeast coast of Brazil where the accidental introduction of *An. gambiae* was reversed by its elimination from an area of approximately 54 000 km^2 *(Soper and Wilson 1943)*. Similar claims were made in the Nile Valley of Egypt (1942-1945) where *An. gambiae* had moved northwards from Sudan and this was reversed by larviciding *(Shousha 1948)*. Though the Brazil campaign against malaria is arguably the most effective in history, its replication in sub-Saharan Africa is doubtful given the ecological differences between Brazil and sub-Saharan Africa. Furthermore, the ability of *An. gambiae* to exploit diverse breeding environments makes the implementation of laviciding programs difficult *(Minakawa et al. 2005)*. WHO recommends the use of larval control in settings where mosquito breeding sites are few, fixed and easily identifiable *(WHO 2012c)*. The Global malaria eradication campaign launched in 1955 with the support from the WHO and the World Bank *(Cohen et al. 2010)* mainly relied on chloroquine for treatment of blood stream infection to reduce clinical illness and the reservoir of human infection and indoor residual spraying (IRS) with the insecticide DDT under the eaves of houses to interrupt transmission by killing the anopheline vectors *(Pambana 1963, Najera et al. 2011)*. This campaign was adversely impacted by the evolution of resistance to both DDT and chloroquine. While the aim of this campaign was to eradicate malaria, the Garki project *(Molineaux 1980)* launched in the early 1970s, aimed at studying the feasibility of mass drug administration and/or

the use of DDT to interrupt transmission. The results revealed that indoor residual spraying with DDT was effective at reducing the ability of the vectors to transmit malaria though was not enough to interrupt transmission in the Sudan savannah of northern Nigeria *(Molineaux 1980)*.

1.7.2 Contemporary malaria control activities.

Recent decades have seen a substantial increase in the resources channeled towards malaria research and control *(Roberts and Enserink 2007)* with the aim of reducing malaria related morbidity and mortality and eventual elimination. These resources have been made available by private foundations (such as the Malaria Foundation, Gates Foundation, Medicines for Malaria Venture, the Wellcome Trust and the Burroughs-Wellcome Fund) *(McCoy et al. 2009)*, international programs and agencies (including the Global Fund, Roll-Back Malaria, the World Bank and the World Health Organization) and national programs such as the Medical Research Council in the UK, the National Institute of Health (NIH), the Centers for Disease Control and Prevention (CDC) and the President's Malaria Initiative (PMI) in the US *(Ceesay et al. 2012)*. Global financing for malaria control programmes increased from an estimated US$ 960 million in 2005 to US$ 2.5 billion in 2014 (Figure 1.6). Of the total invested in 2014, international funding accounted for 78% *(WHO 2015b)*.

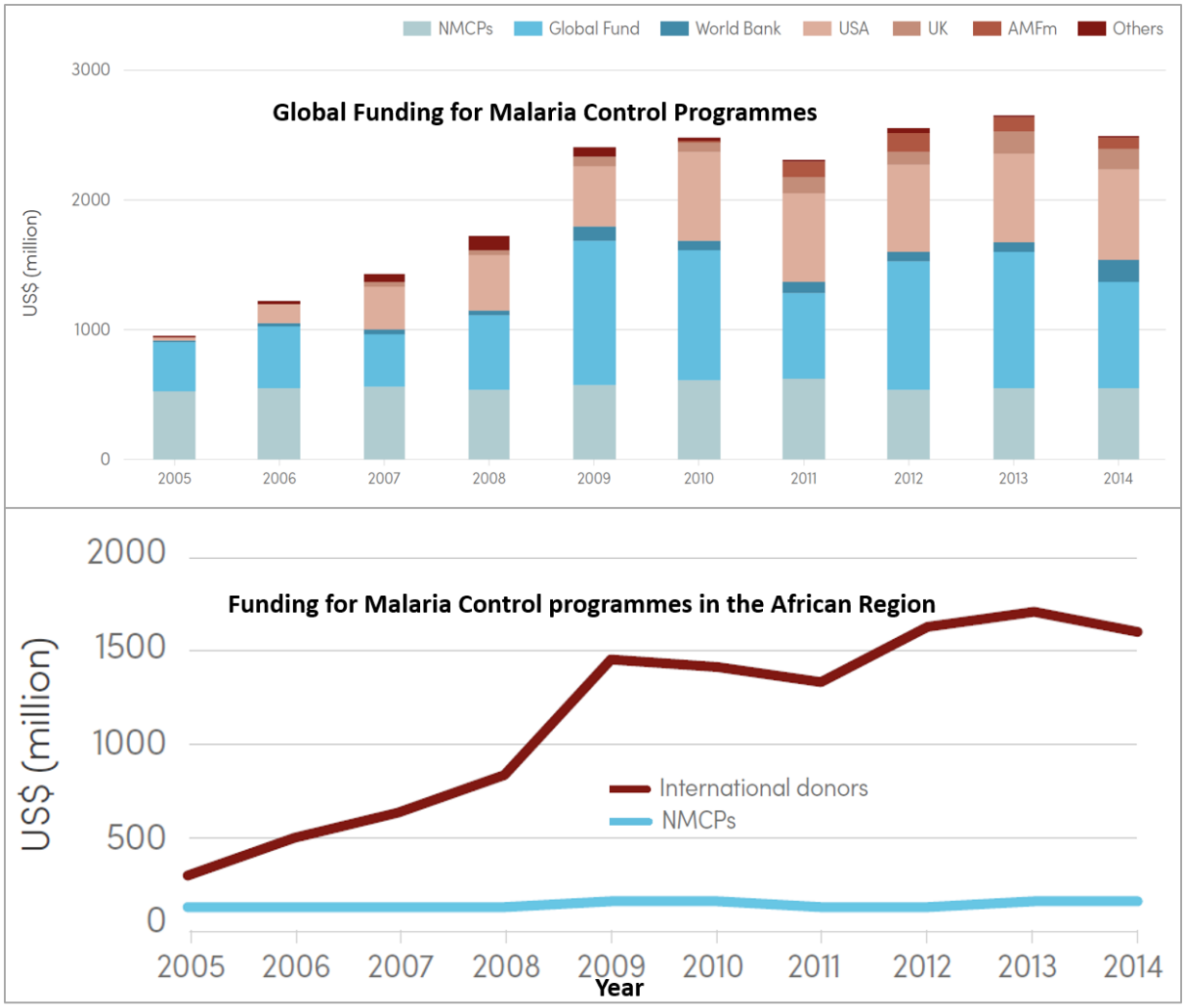

Figure 1.6: Investments in malaria control activities by funding source, 2005–2014.

AMFm, Affordable Medicine Facility-malaria; Global Fund, Global Fund to Fight AIDS, Tuberculosis and Malaria; NMCP, national malaria control programme; UK, United Kingdom; USA, United States of America (WHO 2015b).

The malaria control mechanisms to which these resources have been directed include: (i) Injectable artesunate and Artemsinin-combination therapies (ACTs) for the treatment of persons with severe and symptomatic uncomplicated malaria infections respectively, (ii) interruption of human-vector contact through Insecticide-treated bed Nets (ITNs) to reduce the frequency of malaria transmission from the anopheline vector to humans, (iii) Intermittent Preventive

Treatment with pyrimentamine + sulfadoxine (Fansidar) during pregnancy (IPTp) and infancy (IPTi), and (iv) insecticide residual spraying (IRS) with DDT *(WHO 2012c)*.

Multicenter trials and observational studies have shown that the use of insecticide-treated nets (ITNs) or curtains are effective in reducing childhood malaria-related morbidity and mortality *(Alonso et al. 1991, Alonso et al. 1993, Cheng et al. 1995, D'Alessandro et al. 1995, Binka et al. 1996, Nevill et al. 1996, Fegan et al. 2007)*. For instance, during the first year of the Gambian National Bednet Program the reduction in all-cause mortality among children 1 - 9 years old was reported as ~25% *(D'Alessandro et al. 1995)*. Similarly, all course mortality on the Kenya coast declined by ~33% while hospital admissions for severe malaria declined by ~44% following ITN use *(Nevill et al. 1996)*. ITN use not only provides personal protection against malaria *(Howard et al. 2000, Lengeler 2000)* but also provide communitywide protection by killing infectious mosquitoes that could otherwise cause infections *(Hawley et al. 2003)*. However there have been concerns of pyrethroid insecticide resistance in the recent past *(Ceesay et al. 2010)*.

IRS targeting endophilic vectors, involves careful controlled spraying of insecticides along the inside walls of a home/house. In 2006, WHO recommended the scale-up of IRS using DDT *(WHO 2006a, Kolaczinski et al. 2007)*. Although DDT may be highly effective in reducing malaria prevalence than pyrethroids and other insecticides *(Kim et al. 2012)*, it has been associated with environmental pollution *(Roberts et al. 2000)*. In addition, although reviews report that DDT is safe *(Curtis 2002, Beard 2006)*, toxicity data from animal studies suggest likely long-term negative health effects upon exposure to DDT *(Ben et al. 2001)*. There are 11 other chemicals currently recommended by WHO for use in IRS, including malathion, bendiocarb, lambda-cyhalothrin, and alphacypermethrin *(WHO 2006a)*.

Between the year 2000 and 2015; ITNs, ACTs and IRS accounted for an estimated 68%, 19% and 13% reductions in the prevalence of malaria respectively (Figure 1.7) *(Bhatt et al. 2015b).*

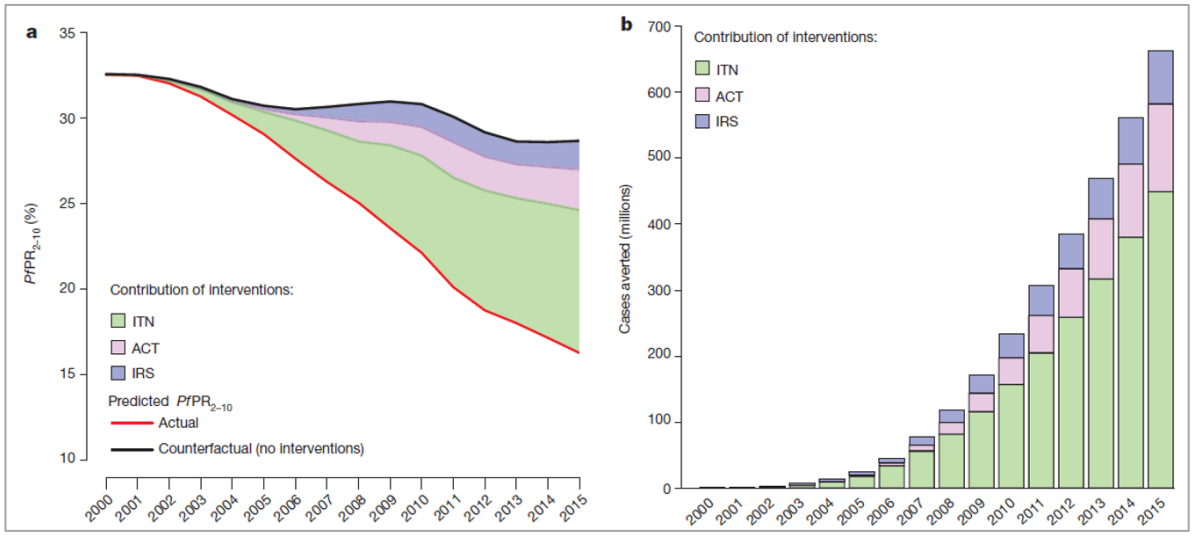

Figure 1.7*: Changing endemicity and effect of interventions 2000–2015.*

*Panel **a** shows the predicted time series of population-weighted mean $PfPR_{2-10}$ across endemic Africa. The red line shows the actual prediction and the black line a 'counterfactual' prediction in a scenario without coverage by ITNs, ACTs, or IRS. The coloured regions indicate the relative contribution of each intervention in reducing $PfPR_{2-10}$ throughout the period. Panel **b** shows the predicted cumulative number of clinical cases averted by interventions at the end of each year, with the specific contribution of each intervention distinguished (Bhatt et al. 2015b).*

IPTp-SP is recommended for all pregnant women in malaria endemic countries starting as early as possible after 1st trimester of pregnancy and given at least one month apart until child birth to improve pregnancy outcomes *(Kayentao et al. 2013, WHO 2013c).* IPTp-SP has been shown to reduce the frequency of placental malaria and its consequence for the newborn *(Schultz et al. 1994).* Although IPTi administered at the time of routine infant immunization was shown to substantially reduce the incidence of malaria and anaemia *(Manzi et al. 2009, Willey et al. 2011),* it has not been adopted as policy for malaria control in East Africa partly due to the shifting

burden of malaria to older children as transmission intensity declines, and partly due to limited resources.

Seasonal malaria chemoprevention (SMC), previously referred to as Intermittent Preventive Treatment in children (IPTc), targeting children less than five years of age has been investigated and recommended as a measure to control the burden of malaria in the Sahel and sub-Sahelian areas of Africa that is characterized by markedly seasonal malaria transmission *(Greenwood 2006)*. SMC involves intermittent administration of full treatment course of amodiaquine plus sulfadoxine/ pyrimethamine (AQ+SP) given to children at monthly intervals, beginning at the start of the transmission season, to a maximum of four doses during the malaria transmission season. The objective is to maintain therapeutic antimalarial drug concentrations in the blood throughout the period of greatest malarial risk to prevent infection. This intervention has been shown to be effective (prevents ~75% of all malaria including severe malaria episodes among treated children), cost-effective, safe, and feasible for the prevention of malaria among children less than 5 years of age in areas with highly seasonal malaria transmission *(Aponte et al. 2009, Wilson 2011).*

Other control methods used in the past few decades include; improved access to treatment by training mothers on proper malaria treatment procedure - this method has been associated with substantial decline in mortality among Ethiopian children *(Kidane and Morrow 2000)*, improved compliance with the treatment through packaging *(Yeboah-Antwi et al. 2001)* and training shopkeepers on the importance of selling a full course of treatment *(Marsh et al. 1999)*.

The possibility of an effective malaria vaccines is being explored *(WHO 2006b)*. Despite decades of intense research and vaccine development effort, there is no commercially available malaria vaccine. Trials targeting the various stages of parasite development have been on the

increase with the hope of identifying a vaccine candidate that can lead to an effective vaccine against malaria. None of the vaccines examined in field trials have attained the vaccine efficacy target of 80% set by the world health organization malaria vaccine technology roadmap *(WHO 2006b)*. RTS,S/AS01 is the most advanced malaria vaccine candidate that has undergone phase 3 evaluation across several sites in Africa. For the 48 months of follow-up, immunization with a fourth dose RTS,S/AS01 vaccine was associated with 36.3% efficacy against clinical malaria among children vaccinated at an older age (5 to 17 months of age) and 25.9% among infants aged between 5 to 12 weeks of age *(RTS 2015)*. The seven-year follow-up data on a three-dose regimen show a rebound suggesting that waning immunity due to vaccination predisposes vaccinated children to more clinical malaria *(Olotu et al. 2016)*. In 2017, The WHO's Strategic Advisory Group of Experts on Immunization and the Malaria Policy Advisory Committee jointly recommended pilot projects to understand how to best implement the malaria vaccine to children in malaria endemic areas *(WHO 2017)*. Kenya, Malawi and Ghana will deploy the vaccine in selected moderate to high malaria transmission settings *(WHO 2017)*. The objectives will be to: a) Evaluate the feasibility of deploying the 4-dose regimen of the RTS,S malaria vaccine through the routine immunization programme, b) consolidate the safety profile of the vaccine when routinely deployed, and c) estimate the impact of the routine delivery of the vaccine on all-cause mortality in children aged 5-48 months *(WHO 2017)*. Given the short term protective effect conferred by the vaccine, the effect will likely be higher in moderate to high transmission settings where children experience multiple episodes of clinical disease per year. Other malaria vaccine candidates are being assessed at various stages of development in addition to other sophisticated malaria control methods that include the development of genetically modified mosquitoes *(De Freece et al. 2014)*.

1.8 Challenges to malaria control programmes

Some of the challenges associated with malaria control include: 1) inequitable distribution of malaria control interventions *(Noor et al. 2007)*, 2) drug resistance as observed in chloroquine, sulphadoxine/ pyrimethamine *(Fryauff et al. 1998)* and now emerging resistance to artemisinin derivatives, 3) pyrethroid insecticides resistance *(Chandre et al. 1999)*, 4) war and civil disturbance leading to increased malaria transmission among refugee populations *(Pitt et al. 1998)*, 5) environmental changes such as farming practices, construction of dams among others *(Ghebreyesus et al. 1999)*, 6) climate change that may have contributed to the spread of malaria to areas that were previously free of malaria due to global warming and floods *(Lindblade et al. 1999)*, 7) travel from endemic areas leading to imported cases of malaria *(Martens and Hall 2000)* and 8) the population increase leading to more people living in endemic areas of the world *(Greenwood and Mutabingwa 2002, Noor et al. 2014)*.

1.8.1 ITN distribution Systems efficiency

Despite huge international investment on ITNs, the processes of delivering and distributing them to where they are most needed are inefficient *(Bhatt et al. 2015b)* and inequitable *(Zegers de Beyl et al. 2016)*. This challenge is further compounded by limited information on the number of ITNs owned and used per household within each country. It is estimated that in 2013, only 43% of people at risk of malaria slept under a net in sub-Saharan Africa. During this period ~21% of new ITNs were allocated to households that already had enough and this inefficient allocation is predicted to have worsened overtime *(Bhatt et al. 2015b)*. Furthermore, nets are much more rapidly lost from households than previously thought. Therefore, systems need to respond to emerging coverage gaps due to continuous nets loss from households resulting from

damage, repurposing, or movement away from target areas and over-allocation *(Bhatt et al. 2015b).*

Gains in ITN coverage have been impressive, currently at ~60% in sub-Saharan Africa, but there remains an enormous challenge towards achieving universal access. Taking measures aimed at more efficient targeting ITNs to households that need them could improve coverage levels to 95% of the total population in need of ITNs *(Bhatt et al. 2015b).* In summary, the rate of net loss and the degree of over-allocation of new ITNs play a major role in determining how delivery will translate into household coverage levels.

1.8.2 Factors influencing ITN use

Key tentative findings from self-reported barriers to mosquito net use within households in sub-Saharan Africa include discomfort primarily due to heat and perceived low mosquito density *(Pulford et al. 2011).* Indoor climate is uncomfortable in the hot-humid zone *(von Seidlein and Knudsen 2016),* furthermore, bednets have been shown to reduce airflow by about 60% *(von Seidlein et al. 2012a).* Therefore, to achieve and maintain high coverage on ITN use, houses should be well ventilated to allow unrestricted airflow. Community engagement and sensitization to integrate housing characteristics that improve ventilation while minimizing vector entry are recommended *(von Seidlein and Knudsen 2016).* Although this effort may take time to implement, it may ultimately expand ITN uptake and consequently improve coverage.

1.8.3 Insecticide resistance

The emergence and spread of insecticide resistance to all four classes of public health insecticides (pyrethroids, organochlorines, organophosphates, and carbamates) threatens the effectiveness of ITNs and indoor residual house spraying. Due to low mammalian toxicity, high speed of action, and high insecticidal activity, pyrethroids are the only insecticide class

recommended by the WHO for use in ITNs *(WHO 2012a)*. Several malaria endemic countries using pyrethroid-based vector control (either impregnated into LLINs or sprayed onto walls) have reported declined effectiveness due to pyrethroid resistance. Nevertheless, in a systematic review and meta-analysis, pyrethroid impregnated ITNs were found to be more effective than untreated nets (UTNs) regardless of resistance *(Strode et al. 2014)*. Further evidence shows that although resistant mosquitoes may not be killed upon exposure to the insecticide, their average life span is considerably decreased *(Viana et al. 2016)*. Continued monitoring of effectiveness with standardized procedures is recommended to guide malaria control programmes *(Strode et al. 2014)*.

1.8.4 Anti-malarial drug resistance

The prospects for malaria elimination are further threatened by the emergency of artemisinin resistance in *P. falciparum* parasites *(Ashley et al. 2014, WHO 2016)*. WHO defines artemisinin resistance as delayed parasite clearance and the presence of PfKelch13 (K13) mutations. As of March 2017, artemisinin resistance had been confirmed in 5 countries of the Greater Mekong Subregion *(WHO 2016)*. Fortunately, majority of the patients in the region still recover following treatment with ACTs *(Ashley et al. 2014, WHO 2016)*. No evidence of wide spread artemisinin resistance has been reported, however, the spread or independent emergence of artemisinin resistance in other parts of the world would be disastrous for the global malaria control programmes given that no alternative antimalaria medicines are available at present with the same level of efficacy and tolerability as ACTs *(WHO 2016)*. In Africa, non-synonymous K13 mutations are still rare and highly diverse, and do not appear to be linked to resistance (Figure 1.8) *(Ashley et al. 2014)*. Resistance to artemisinin products may have resulted from a combination of factors that include: poor treatment practices, poor adherence of the complete

course of treatment, widespread availability of oral artemisinin-based monotherapies and substandard generic drugs in the market.

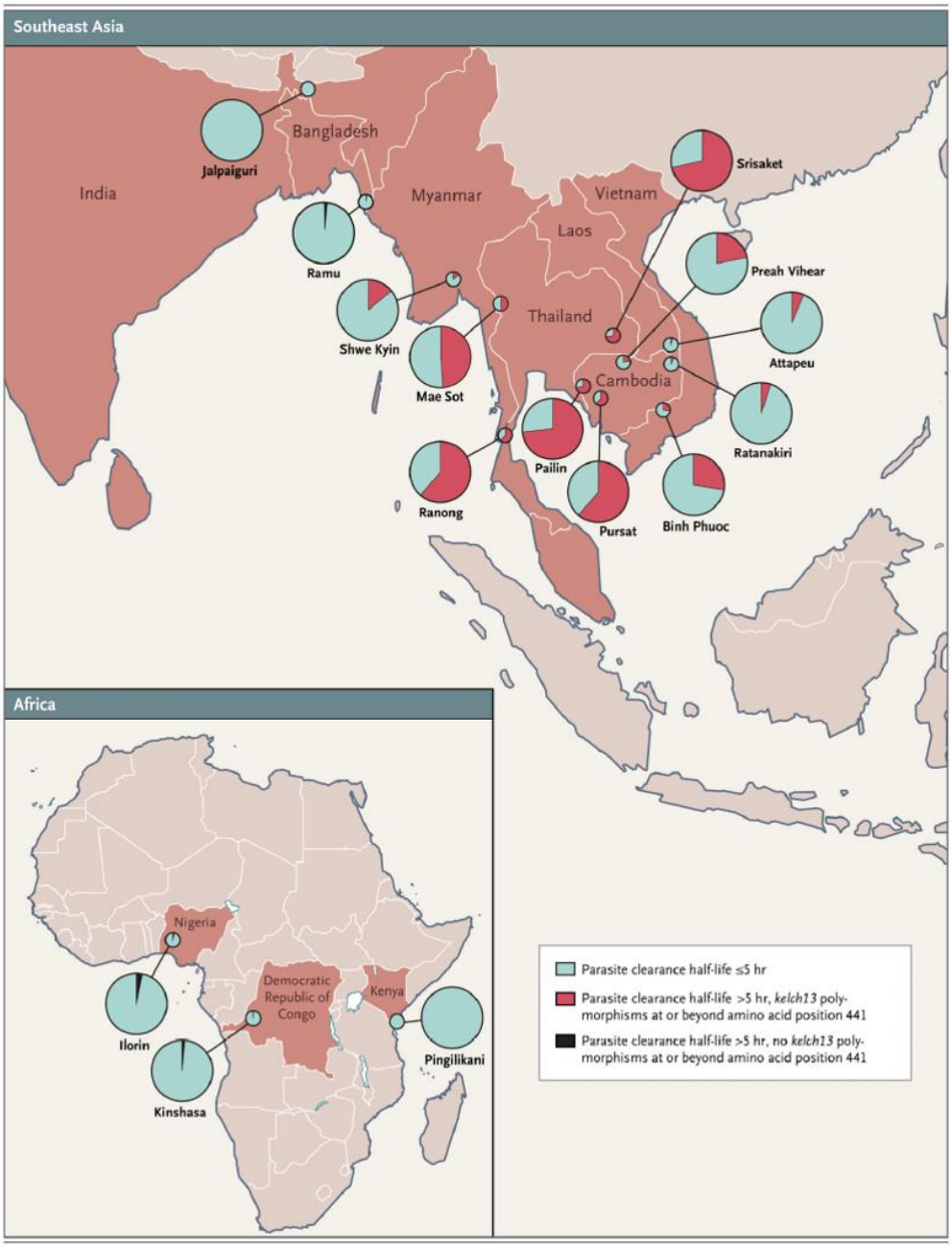

Figure 1.8*: Location of study sites and proportions of patients with artemisinin resistance.*

Artemisinin resistance was defined by a parasite clearance half-life longer than 5 hours, with some Plasmodium falciparum isolates having kelch13 polymorphisms (at or beyond amino acid position 441) (Ashley et al. 2014).

1.9 Malaria case detection methods

Active case detection (ACD) involves detecting malaria infections by community health workers at the community or household level. Active case detection can be done for clinical malaria or for the acquisition of infection. The former involves regular fever screening of a longitudinal cohort followed by parasitological examination of all febrile patients for symptomatic malaria. The latter would involve giving anti-malarials to clear parasitaemia at baseline, followed by regular parasitological examination of the full cohort without prior fever screening *(Sturrock et al. 2013)*. Passive case detection (PCD) on the other hand, entails screening febrile patients presenting to health care facilities for malaria parasites. PCD detect symptomatic malaria cases but also include other febrile illness with coincident malaria infection *(Sturrock et al. 2013)*. This method misses asymptomatic parasitaemia cases present in the community who typically do not seek medical care. The sensitivity of detection for both ACD and PCD is limited to the diagnostic tool used *(Okell et al. 2009, Okell et al. 2012)*.

1.10 Interventions targeting asymptomatic reservoir

Two common interventions targeting asymptomatic parasitaemia exist; 1) mass drug administration (MDA) and 2) mass screen and treat (MSAT).

The most inclusive way to clear the asymptomatic reservoir of malaria infections is through mass drug administration (MDA) *(Okell et al. 2011)*. MDA involves administering a full dose of anti-malaria chemotherapy to the population with no prior screening. Although this method is logistically demanding, its application ensures that submicroscopic infections are also targeted and is effective in low transmission settings *(Bousema et al. 2012b, Sturrock et al. 2013, Newby et al. 2015)*.

To achieve high local coverage, mass screening and treatment (MSAT) strategies are used. Anti-malarial drugs that target both asexual parasites and gametocytes are recommended in addition to vector control. Giving antimalarial drugs specifically based on positive test results may be preferable to presumptive treatment (MDA) since such a strategy limits excess drug use that may drive antimalarial resistance. However, the insufficient sensitivity of existing field diagnostics commonly used to identify asymptomatic infections makes MDA justifiable. Furthermore, studies have shown that MSAT has limited effect in reducing transmission *(Cook et al. 2015).*

1.11 Measuring transmission intensity

With renewed interest in malaria elimination, detailed understanding of malaria transmission intensity in space and time is critical. Measuring malaria transmission intensity is a key element of monitoring changes in transmission and assessing the impact of anti-malaria interventions. Cost effective and accurate measurement of transmission intensity is required to provide helpful insights on when and where the greatest risks occur and thus guide the development and implementation of appropriate control strategies *(Smith et al. 2005).* In sub-Saharan Africa, malaria transmission intensity varies substantially, a feature associated with the vectorial capacity of the local mosquitoes, human acquired immunity and the malaria control interventions in place *(Smith et al. 2010).* Several ways of measuring transmission intensity are described in the literature. The commonly used metrics discussed here are: (1) Parasite rate in humans, (2) malaria positive fraction (MPF) or slide positive rate (SPR), (3) entomological inoculation rate, (4) force of infection and molecular force of infection, (5) multipliscity of infection, (6) seroconversion rate, (7) vectorial capacity and the basic reproduction number, and (8) net infectiousness of humans to mosquitoes.

1.11.1 Parasite prevalence in humans (PR)

PR is defined as the proportion of individuals that are parasitaemic at a given point in time *(Tusting et al. 2014)*. Although consistently referred to parasite rate in the literature to date, here I refer it to parasite prevalence and not parasite rate per se. PR is estimated in cross-sectional surveys using RDTs, microscopy or PCR *(Wu et al. 2015)*. This metric may not be definitive when applied to asymptomatic individuals since parasite densities fluctuate above and below threshold of detection over the course of infection *(O'Meara et al. 2007)*. Similarly, detection thresholds vary with method used (i.e. RDT, light microscopy or PCR) *(Bejon et al. 2006, O'Meara et al. 2007)*. The suitability of using PR for quantifying transmission intensity has been criticized in literature since it is not a direct measure of transmission intensity and becomes saturated at high transmission intensities *(Smith et al. 2005, O'Meara et al. 2007)* due to heterogeneous biting, multiple infection and acquired immunity. The measure should also be age corrected for standardization across sites *(Smith et al. 2007a)*.

1.11.2 Malaria positive fraction (MPF)

MPF is also referred to **Slide positive rate (SPR)** in the literature, and is defined as the proportion of children presenting with fever to a healthcare facility who test positive for malaria parasitaemia by either light microscopy or RDT. I will use MPF as my preferred term over SPR since the measure is a fraction and not a rate per se *(Bejon et al. 2014)*. MPF is measured through passive or active case detection methods especially at health facilities where routine screening is done using RDTs and/or microscopy *(Tusting et al. 2014)* or through surveys in the community *(Hawley et al. 2003)*. Though the accuracy of MPF is not well established, it has previously been used to characterize transmission intensity in Sub-Saharan Africa *(Ceesay et al. 2010)* and used to explain variations in incidence of malaria *(Bi et al. 2012)*. MPF is useful

as a rapid indicator of trends and is thus recommended by the WHO when measuring changes in transmission intensity *(WHO 2010)*. In addition, MPF has been shown to have a positive association with malaria incidence in some studies *(Bi et al. 2012)*. When data are collected through active case detection the incidence rates can be computed with minimal biases. However, when data are collected through passive case detection, incidence rates are prone to biases due to, for instance, accessibility to hospital *(Tusting et al. 2014)*. For this reason, MPF is preferred as it is less prone to bias due to distance from hospital *(Bejon et al. 2014)*. That is, access to hospital equally affects the slide positive and slide negative children residing in the same locality hence normalizing the effect of access to care *(Bejon et al. 2014)*.

1.11.3 Entomological inoculation rate (EIR)

EIR is a metric that estimates the number of bites per person per unit time (mostly in years) and is therefore a measure of exposure to infectious mosquitoes *(Ceesay et al. 2010, Kilama et al. 2014, Tusting et al. 2014)*. It is estimated by catching mosquitoes and then dissecting them to estimate the sporozoite rate *(Birley and Charlewood 1987)*. Human biting rates (Ma) are estimated by catching and counting the number of mosquitoes that attempt to feed on a human while sporozoite rate (SR) is found by examining those mosquitoes for the presence of sporozoites *(Smith et al. 2005, Kilama et al. 2014)*. Standard methods proposed for measuring Ma include human landing catches, pyrethroid spray catches, exit traps and CDC light traps *(WHO 2013b, Kilama et al. 2014)*. This metric has several limitations; 1) most EIR studies provide little or no explanation on the rationale behind the choice of locations, houses, or trap placements, information on the human collectors, time of day, or frequency of mosquito collections. 2) EIRs are often poorly reproducible in practice, very laborious to collect and results vary within methods *(Kelly-Hope and McKenzie 2009)*. EIR values tend to be higher

than values of force of infection (FOI), because not all infectious mosquito bites result in an infection *(Churcher et al. 2017)*. Additionally, sporozoite rates, and by extension EIRs, are affected by seasonal variations and lack sensitivity at low transmission settings *(Kelly-Hope and McKenzie 2009)*.

1.11.4 Force of infection (FOI) and molecular force of infection (mFOI)

FOI is defined as the number of infections per person per unit time, and the mFOI is the number of new parasite clones acquired per unit time *(Mueller et al. 2012)*. FOI can be measured through cohort studies (which may be naturally uninfected, artificially created cohorts) or repeated cross-sectional surveys *(Smith et al. 2010)*. The limitation resulting from previously acquired infections can also be mitigated by either assessing infant conversion rates which requires monitoring many infants over long period, or by using the mFOI *(Mueller et al. 2012)*. This metric (mFOI) allows the measurement of transmission intensity without the need to clear previous infections in longitudinal studies *(Mueller et al. 2012)*. Both FOI and mFOI suffer from accuracy that is as a result of parasite fluctuation within infected persons, especially when they fluctuate below the detection limit of the commonly used diagnostic tools *(Koepfli et al. 2011)*. The estimates of mFOI is also affected by seasonality, age and some malaria control methods *(Mueller et al. 2012)*. Compared to FOI, mFOI has greater sensitivity and specificity and has a relatively high precision and accuracy in low transmission areas, and continues to show variation when FOI has saturated *(Tusting et al. 2014)*.

1.11.5 Multiplicity of infection (MOI)

MOI is the number of concurrent parasite clones per *P. falciparum*-positive host *(Tusting et al. 2014)*. MOI is determined by genotyping parasites using polymorphic markers such as the merozoite surface protein-1 (MSP-1) *(Atroosh et al. 2011)* or merozoite surface protein-2 (MSP-

2) *(Mueller et al. 2012)* amongst others *(Tusting et al. 2014)*. The capacity of MOI to accurately reflect transmission intensity may depend on both the diversity of the parasite clones and the frequency of sampling *(Koepfli et al. 2011)*. It has been demonstrated that in areas where parasite populations are less diverse, transmission intensity may be underestimated as parasite clones may not be distinguishable by molecular markers *(Mueller et al. 2012)*. However, higher numbers of *P. falciparum* concurrent infections (mean = 5) have been detected in highly endemic areas of sub-Saharan Africa *(Beck et al. 1997)* while low MOI ranging between 1.5 to 1.7 detected in Papua New Guinea, an area that is relatively a low transmission zone *(Koepfli et al. 2011)*.

1.11.6 Seroconversion rate (SCR)

Anti-malarial antibodies are biological markers of previous infection that can explain spatial-temporal trends in transmission intensity *(Drakeley et al. 2005, Bousema et al. 2010b)*. Since antibodies are raised by the host in response to infection, serology is thought to be a good indicator of exposure to malaria. Serological tools are potentially more sensitive and robust than parasite prevalence or EIR given that antibodies last longer than patent parasitaemia and longer than the lifespan of infective mosquitoes. In the past, serological tools have been used to examine the impact of interventions that reduce malaria exposure *(Cornille-Brogger et al. 1978)*. Some commonly targeted malaria antigens used in serological studies include a) circumsporozoite protein (CSP), b) merozoite surface protein-1_{19} (MSP-1_{19}) and c) apical membrane antigen-1 (AMA-1). CSP responses are not rapidly acquired, and are therefore not suitable for estimating transmission intensity in low transmission settings. MSP-1_{19} is moderately immunogenic and can be used to estimate changes in transmission intensity over a range of transmission intensities. AMA-1 on the other hand is highly immunogenic and can

elicit long-lived immune responses that can lead to saturation at moderate transmission intensities *(Drakeley et al. 2005)*. Serological measures of malaria infection have been proposed for diagnosis and determination of community level transmission in the pre-elimination and elimination phases of malaria control *(malERA 2011)*. Seroconversion to malaria is the development of detectable anti malaria antibodies against the parasite antigen in the blood *(Drakeley et al. 2005)*. The incidence of seroconversion can be calculated by fitting a reverse catalytic model to age-specific malarial seroprevalence data *(Drakeley et al. 2005)*. SCR estimates analogous to force of infection can also be computed *(Drakeley et al. 2005, Corran et al. 2007)*. A further advantage of using SCR as a measure of transmission intensity is its ability to account for malaria exposure over time, hence allowing temporal patterns in transmission intensity to be studied *(Corran et al. 2007)*. This metric has recently gained popularity as a tool for monitoring variations in malaria transmission intensity in Africa *(Corran et al. 2007, Stewart et al. 2009)*, Asia *(Lim et al. 2005)* and the Pacific *(Cook et al. 2010)* where studies have shown close correlation between independent metrics of malaria transmission intensity and SCR. Although this metric has been associated with high precision and accuracy, SCR may not be sensitive to short-term changes in transmission given that antibodies can persist for years after the exposure period *(Corran et al. 2007)*, however, this weakness can be mitigated by assessing seroconversion among children under five years of age *(Ceesay et al. 2010)*.

1.11.7 Vectorial capacity (C) and the basic reproduction number (Ro)

C is defined as the expected number of infectious bites that could eventually arise from all the mosquitoes that bite a single human on a single day assuming non immunity and 100% efficiency in transmission *(Garrett-Jones 1964)*. It is mathematically denoted as

$$C = \frac{\mathrm{M}a^2p^n}{-ln\ \mathrm{p}},$$

where M is the density of adult mosquitoes, a is their feeding frequency on humans, p is their daily survival rate and n is the duration of parasite development in humans.

The malaria basic case reproduction number R_0 is the expected number of hosts who would be infected after one generation of the parasite by a single infectious person who had been introduced into an otherwise naive population. It is defined as the product of vectorial capacity, the net efficiency of transmission and the duration of the infection *(Johnston et al. 2013)*. The estimation of C from the individual components is normally difficult and prone to compounded error that result from the errors introduced by individual terms *(Dye and Hasibeder 1986)*. To sustain transmission a minimum value ($R_0 = 1$) must be attained. Though R_0 remains a gold standard for measuring transmission intensity, it is difficult to measure accurately in the field *(Tusting et al. 2014)*.

1.11.8 Net infectiousness of humans to mosquitoes (k)

At the population level, k is defined as the fraction of mosquitoes that would become infected after blood feeding on any human. The determination of k is normally influenced by processes acting in both humans and mosquitoes and includes: fluctuations in gametocyte density, naturally acquired transmission-blocking immunity *(Tusting et al. 2014)*, the efficiency with which the gametocytes are taken up by mosquitoes in a blood meal relative to their measured density, the effective contact of the vectors with humans and factors influencing the susceptibility of mosquitoes to malaria infection such as the mosquito innate immunity *(Trape et al. 1987, Smith et al. 2004)*, midgut natural microbiota *(Cirimotich et al. 2011)* among others. In practice, it is impossible to directly measure this metric in a natural mosquito population.

However, the metric can be estimated using; (1) direct skin feeding assays (SFAs) where laboratory-reared mosquitoes are directly fed on infected humans to determine the proportion that become infected *(Killeen et al. 2006)*, (2) using standard membrane-feeding assays (MFA) *(Bousema et al. 2012a)* and (3) infection rates in the natural vector population *(Burkot et al. 1990)*. Though k has been shown to correlate well with EIR at low transmission areas, it has been criticized for its low precision and accuracy *(Tusting et al. 2014)*. To measure the effect of sexual-stage transmission-blocking vaccine, accurate measurement of k is needed before and after vaccination.

1.12 Spatial and temporal heterogeneity of malaria

Plasmodium falciparum transmission is the process by which a malaria parasite completes its life cycle from human to human via a mosquito vector *(Tusting et al. 2014)*, and the intensity of transmission refers to the frequency of new human infections *(Smith et al. 2012)*. Transmission intensity for most infectious agents (including *Plasmodium falciparum* malaria parasites) is heterogeneous as has long been recognized empirically and explored using mathematical models. For many infectious diseases, ~20% of the human population account for ~80% of the infectious burden *(Woolhouse et al. 1997b)*. The processes leading to malaria transmission depend on the ecology and biting behavior of mosquitoes which in turn is determined by climate (rainfall, temperature and humidity), hydrology and soil composition *(Packard and Gadehla 1997, SHILILU et al. 2003, Olson et al. 2010, Stefani et al. 2013, Weiss et al. 2014)*, the distribution of human residence and behaviors (urbanization and housing) *(Hay et al. 2005, Omumbo et al. 2005)*, control interventions *(Bhatt et al. 2015a)* altitude *(Brooker et al. 2004, Ernst et al. 2006, Baidjoe et al. 2016)*, proximity to dense vegetation *(Ernst et al. 2006, Kreuels*

et al. 2008), wind direction *(Midega et al. 2012),* and host genetic factors *(Mackinnon et al. 2005).*

Rainfall combined with suitable temperatures (between 25ºC and 30ºC) provides optimal breeding environment for the vector mosquitoes while humidity is associated with vector longevity *(Dutta and Dutt 1978).* High temperatures of (>34ºC) are associated with high larval and adult mosquito mortalities *(Kirby and Lindsay 2004)* while temperatures below 16ºC are unable to produce viable adults *(Bayoh and Lindsay 2003, Bayoh and Lindsay 2004)*. Irrigation schemes, dams and swampy areas provide suitable breeding ground for mosquitoes to oviposit *(Ijumba and Lindsay 2001)* thus increasing mosquito abundance. On the other hand, aridity negatively affect the vector development and survival *(Shililu et al. 2004)*. Limited availability of water bodies reduces the number of suitable sites for oviposition and also reduces the mosquito survival rate at all stages of development *(Gray and Bradley 2005).*

Social economic status and housing characteristics are key determinants of malaria transmission intensity *(Ernst et al. 2006, Tusting et al. 2013, Tusting et al. 2015, von Seidlein and Knudsen 2016).* Improvements to housing contributes significantly to malaria control and elimination by decreasing house entry by malaria infected mosquitoes and thus exposure to biting. Housing quality is an important risk factor for malaria infection across the spectrum of malaria endemicity in sub-Saharan Africa *(Tusting et al. 2015, Tusting et al. 2017).* Similarly, lower economic and/or education levels are associated with increased risk of malaria transmission *(Baragatti et al. 2009, Tusting et al. 2013).*

Urban areas have lower malaria incidence compared to rural areas of sub-Saharan Africa, this is partly due to limited availability of optimum environmental conditions for the development of the vector mosquitoes thus resulting in low vector densities and low transmission intensity *(Trape et al. 1987).* In addition, accessibility to anti-malarial drugs and modern housing in urban

settings provide a plausible explanation for the spatial variation observed between rural and urban settings. Inequitable distribution of malaria control programs in rural settings has been documented *(Noor et al. 2007)*. Malaria control programs tend to distribute control interventions to areas that are more accessible hence leaving out areas that need the nets most.

The temporal variations of malaria transmission witnessed in sub-Saharan Africa have also been attributed to climate change *(Afrane et al. 2012)* (for instance, the highlands of Kenya have experienced increasing malaria transmission), increased urbanization resulting from increasing population density, scaled up malaria control interventions, increased irrigation schemes among others. These factors act at various spatial scales and may explain why some households, villages, regions, countries and continents experience higher risk of malaria while others remain free or experience fewer episodes of the disease.

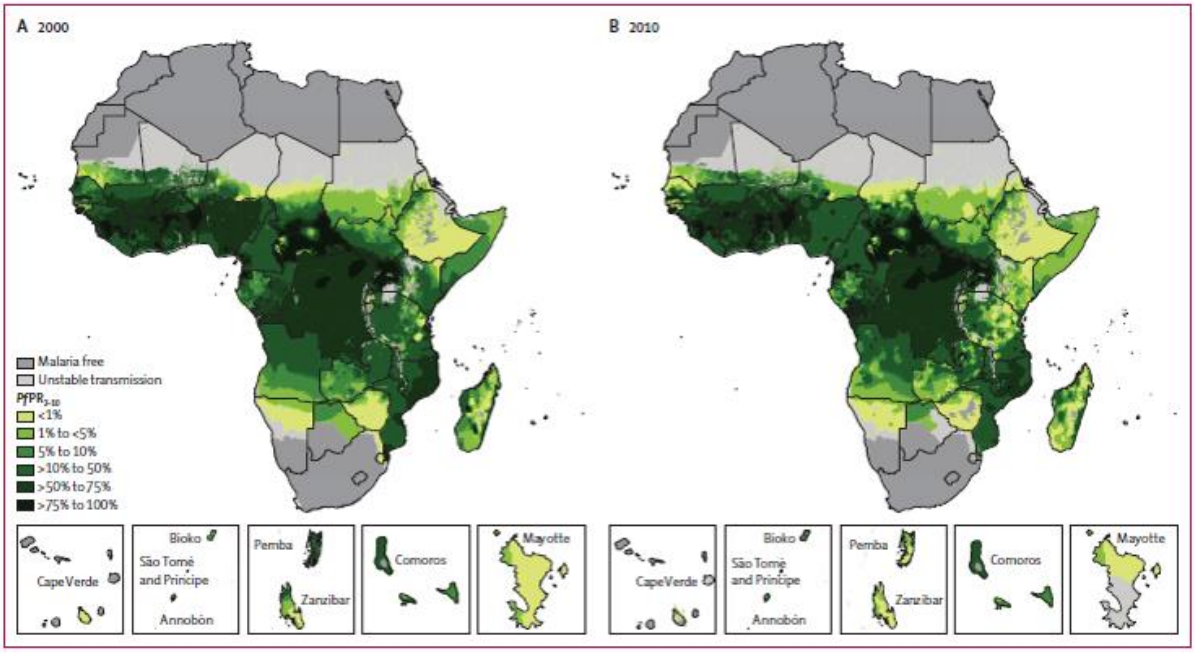

Figure 1.9*: Predicted 1 × 1 km spatial resolution Plasmodium falciparum parasite rate endemicity class maps of Africa prevalence in (A) 2000 and (B) 2010.*

The dark grey, light grey, and white areas are malaria free and the limits of unstable and stable transmission, respectively, for each year. Pf*PR*$_{2–10}$ *predictions were made to areas within the stable limits of transmission.* Pf*PR*$_{2–10}$*=community Plasmodium falciparum parasite rate standardised to the age group 2–10 years (Noor et al. 2014).*

Just as there are variations in the determinants of malaria transmission at various spatial scale, malaria heterogeneity is apparent at a global scale *(Noor et al. 2014)*, Figure 1.8, with some continents (for instance Africa) experiencing high transmission while others experience very low to no transmission. Similarly there are variations within continents - where transmission ranges from holo-endemic to meso-endemic or even no transmission *(Noor et al. 2014)*, and further variations have been observed within countries, for instance, Mali *(Gaudart et al. 2006)*, Ghana *(Kreuels et al. 2008)*, Ethiopia *(Yeshiwondim et al. 2009)*, Kenya *(Brooker et al. 2004, Bejon et al. 2010)* and Tanzania *(Bousema et al. 2010a)* and between homesteads within the same village*(Bejon et al. 2014).*

1.13 Remote sensing

Modelling malaria transmission is often challenging. The identification of all the natural factors (such as seasonality, rainfall, temperature, humidity, surface water and vegetation) and anthropogenic elements (such as agriculture, irrigation, deforestation, urbanization and movement of populations) that likely correlate with transmission intensity is logistically difficult and expensive *(Stefani et al. 2013)*. The ultimate aim is usually to determine malaria incidences without clinical surveillance and thus enhance the identification of potential at risk areas at various spatial scales *(Bannister-Tyrrell et al. 2017)*. Data collection techniques that monitor environmental changes relevant to the epidemiology of infectious diseases have gained popularity in the recent past *(Tatem et al. 2004)*. For instance, remote sensing (RS) (i.e., the process of acquiring information about an object area or feature from a distance) *(Hay 2000)*

has been used extensively to study environmental differences such as land cover, altitude and climatic conditions *(Kulkarni et al. 2010)*. The use of RS to provide new insights for malaria studies has been reviewed extensively *(Rogers et al. 2002, Herbreteau et al. 2007)*. From the review by Herbreteau et al. *(Herbreteau et al. 2007)*, RS is often, and increasingly, used to study parasitic diseases (59% of studies) including malaria (16% of studies). The distribution of malaria has been shown to depend on static geographical factors as well as environmental alterations over time *(Stefani et al. 2013)*. Ecosystem changes resulting from natural features or human interventions, on a local or global scale, can alter the ecological balance and context in which vectors and their parasites develop and transmit disease *(Patz et al. 2000)*. The mosquito population processes and malaria incubation periods in vectors have been shown to vary with temperature and moisture conditions *(Kulkarni et al. 2010)*. Similarly, human interventions such as forest clearance and pollution have been associated with changing malaria transmission rates *(Conn et al. 2002, Patz and Olson 2006, Olson et al. 2010)*. Curran et al. *(Curran et al. 2000)* propose the linking of malaria incidence with land cover (LC) and/or land use (LU) characteristics while Ostfeld et al. *(Ostfeld et al. 2005)* propose the use of more explicit landscape approaches to study eco-epidemiological systems that may improve the understanding and prediction of the malaria risk.

1.14 Hotspots

1.14.1 Defining a hotspot of malaria transmission

WHO defines a focus of malaria transmission as a defined and circumscribed locality situated in a currently or formerly malarious area containing the continuous or intermittent epidemiological factors necessary for malaria transmission *(WHO 2007)*. A hotspot of malaria transmission is defined as a geographical part of a focus of malaria transmission where

transmission intensity exceeds the average level. Therefore, several hotspots of malaria transmission may be present in a single focus of transmission.

1.14.2 Importance of studying hotspots of malaria transmission

Hotspots fuel transmission within transmission foci, whereas the foci form independent malarious areas with minimal or no transmission between them, and may themselves contain hotspots. Two main reasons why hotspots are relevant for malaria control have been proposed *(Woolhouse et al. 1997b, Smith et al. 2007b)*. Firstly, on theoretical grounds, if interventions are untargeted, hotspots are likely to be the areas where residual malaria transmission will persist. This hypothesis is supported by observations made in the field *(Bautista et al. 2006, Ernst et al. 2006)*. Hotspots of malaria transmission can thereby form a major stumbling block in efforts to eliminate malaria *(Moonen et al. 2010)*. Secondly, hotspots of malaria transmission are likely to play a catalyzing role in areas of stable transmission *(Bousema et al. 2010a)*. Interventions targeted at transmission hotspots have the potential to reduce community-wide malaria transmission intensity *(Bousema et al. 2010a)*. Thirdly, if resources are limited then they should be directed on those most at risk.

1.14.3 Potential for targeted control

Malaria hotspots maintain malaria transmission during the low transmission seasons and thus may provide the parasite reservoir during the high transmission season *(Bousema et al. 2012b)*. Elimination strategies may need to focus reactive screening and treatment of households in endemic zones *(Moonen et al. 2010)*. The procedure can be enhanced through proactive case detection, where people living within hotspots are screened for parasitaemia and treated at regular intervals *(Moonen et al. 2010)*. Targeted control would mean that the most diseased households are prioritized *(Moonen et al. 2010, Stresman et al. 2010, Bousema et al. 2012b)*,

thus reducing the transmission intensity to the whole population. Detecting hotspots involves mapping asymptomatic carriers through cross sectional surveys, hospital surveillance for symptomatic parasitaemia, or using serological tools *(Moonen et al. 2010)*. Targeting households within a hotspot is likely to be logistically feasible, much more efficient, and may allow for more complicated interventions than if hotspots were untargeted.

1.14.4 Challenges with hotspot targeted control

The topic of targeting hotspots of malaria transmission has gained interest in the recent past *(Bannister-Tyrrell et al. 2017)*. In a cluster, randomized trials targeting hotspots in western Kenya, modest results were achieved inside the hotspots but no lasting results were observed outside the hotspots *(Bousema et al. 2016)*. Important implementation questions remain; 1) to what extent are hotspots of transmission stable and generalizable? *(Bejon et al. 2010)* 2) at what level of transmission intensity should hotspots be targeted to achieve interruption? 3) are hotspots detected by different diagnostic tools consistent? 4) How do we define hotspots boundaries, and sample size? *(Stresman et al. 2017)* These parameters need to be well understood to aid the design of an effective targeted intervention programme.

1.15 Scope

The proposed scope of this work comprise a study of febrile malaria children admitted to the Kilifi county referral hospital over a 25-year period (1990 - 2014). Two cohorts of children within the KHDSS area followed actively for febrile malaria was used to validate the use of malaria positive fraction at KCH as a proxy measure of community incidence rate. Politically defined geographical areas (locations) linked to clinical data allowed a 25-year analyses of age, spatial and temporal variations of admissions with malaria from the KHDSS area, whereas the more comprehensive KHDSS data collection from the year 2003 allowed a more detailed

analysis of homestead level variations using the geographical coordinates system. From the year 2009, yearly data collection on ITN use for all residents of the KHDSS and remote sensing data downloaded from the year 2000 to 2014 allowed detailed analysis of the effectiveness of personal and community ITN use, and the effect of soil moisture on the probability of admission with malaria. Work on the multicenter micro-epidemiological analyses was an important extension following earlier observations that heterogeneity becomes more apparent as transmission intensity declines. The collated datasets from studies conducted in areas experiencing variable transmission intensities in sub-Saharan Africa allowed for a detailed secondary analysis of various micro-epidemiological parameters and their association with overall transmission intensity. Whereas studies of febrile malaria may be less affected by the diagnostic tool used given the accompanying high parasite biomass, studies of asymptomatic parasitaemia are likely to be more sensitive to the diagnostic tools used especially in areas of low transmission intensity. Work on the detection of asymptomatic malaria hotspots by diagnostic tool (PCR, microscopy and RDT), allowed an assessment of the degree with which hotspots detected by microscopy and RDT overlap with those detected by PCR, and a further comparison of the stability of hotspots among diagnostic tools.

1.16 Composition of the thesis

I will introduce the specific datasets and their contextualization in the specific chapters describing the results. Chapter 2 will focus on the statistical methodology common to all chapters. Thereafter the main data chapters are:

Chapter 3. An analysis of the age, spatial and temporal variations in admissions with malaria to Kilifi county hospital and the assessment of the factors predicting admissions with malaria parasites.

Chapter 4. A descriptive analysis of micro-epidemiology of malaria transmission intensity using multicenter datasets assembled from sub-Saharan Africa.

Chapter 5. A comparison of malaria hotspots detected by Microscopy, RDT and Polymerase Chain Reaction

Each chapter contains a brief introduction, data description, additional analysis specific to that chapter, results and discussion. Chapter six contains the overall discussion of the findings, further areas of research and conclusions.

Chapter Two

2 Material and Methods

2.1 Spatial analysis

Identification of disease clusters is essential for public health surveillance and potential targeting. Spatial analyses to describe clustering have been extensively applied in malaria epidemiology *(Pullan et al. 2012)*. For instance, Kulldorff's spatial scan statistic and the Moran's I statistic have been used in malaria epidemiology to describe spatial heterogeneity *(Brooker et al. 2004, Ernst et al. 2006, Bejon et al. 2010, Bousema et al. 2010a, Bejon et al. 2014)*. The K function has been applied to investigate spatial clustering of households with seropositive children during evaluation of targeted screening strategies to detect Trypanasoma cruzi infection *(Levy et al. 2007)* and the identification of malaria clustering and hotspots in an endemic area in western Kenya *(Wanjala et al. 2011)*, among other applications. The scan statistic identifies hotspots that would potentially benefit from targeted intervention *(Bousema et al. 2016)*, while the Moran's I and the Variogram are used to examine spatial auto-correlation in certain continuous features of malaria transmission.

Methods for local cluster detection include; the Geographical Analysis machine (GAM) also referred to Openshaw *(Openshaw et al. 1987)*, Anselin's local indicator of spatial association (LISA) *(Anselin 1995)*, the Besag-Newell test *(Besag and Newell 1991)*, the spatial scan statistic among others. I used the Kulldorff's spatial scan statistic to detect local clustering because it adjusts for multiple testing, heterogeneous population density distributions and potential confounders.

Kulldorff's spatial scan statistic *(Kulldorff 1997)*, estimated in SaTScan™ software (http://www.satscan.org), was used to detect hotspots of symptomatic malaria and

asymptomatic parasitaemia. SaTScan imposes a circular scanning window that moves across geographical space with radius varying from zero to a maximum radius enclosing at most 30% (pre-specified by the user) of the population in the sampling frame. For each location and size of the window, the number of observed cases are counted and the expected cases computed by assuming a uniform distribution of cases across the population. The scan statistic compared the count within each circle and that outside to derive a log likelihood statistic. The testing procedure for the null hypothesis of complete spatial randomness, is based on the Monte Carlo simulations where for each simulation run, the observed cases across the entire set of data locations are randomly permuted in space according to the data locations and population size. The expected log likelihood under random simulations is then computed. The observed log likelihood is then compared with the simulated log likelihoods to determine statistical significance.

An elliptic window shape can be used as an alternative to the circular window, in which case a set of ellipses with different shapes and angles are used as the scanning window. This may provide higher power for true clusters that are elliptical in shape, but lower power for circular and other very compact clusters, and comes with extra computational cost.

I restrict my analysis below to using circular windows to detect hotspots. This may not be realistic in some settings, for instance where hotspots follow a river and could well be elliptical. Circular hotspots on the other hand are the most commonly used in practice *(Bannister-Tyrrell et al. 2017),* they are computationally efficient and it is easier to determine hotspots properties that are of interest (Radius, Risk Ratio and significance).

2.2 Spatial functions

Several techniques for examining disease clustering have been described in literature *(Auchincloss et al. 2012)* including: the kernel intensity function *(Kelsall and Diggle 1995)*, Cuzick and Edwards' method *(Cuzick and Edwards 1990)*, the Knox test *(Kulldorff and Hjalmars 1999)* and the K-function *(Ripley 1977)*. A disadvantage with the kernel intensity ratio is the preselection of a bandwidth that can lead to variation in results depending on the selected bandwidth *(Wheeler 2007)*. Similarly, Cuzick and Edwards' method requires pre-selection of the number of nearest neighbours *(Wheeler 2007)*, while the knox test requires a prior choice of critical distances to determine which pairs of cases are close in space and time *(Kulldorff and Hjalmars 1999)*. These preselected parameter choices are mostly subjective and at best dependent on prior knowledge and may thus influence statistical inference. The K-function was preferred for the analysis of binary data because it corrects for edge effects, does not require prior specification of parameters that may influence statistical inference and allows for a range of spatial and temporal scales. Other spatial functions commonly used in spatial point pattern analysis for continuous data include the Variograms and Moran's I *(Pullan et al. 2012)*. Here, the Moran's I and the *K* functions were used to examine global spatial dependence in malaria transmission.

Ripley's *K*-function for a distance *d*, *K(d),* is a second-order analysis of point patterns in two-dimensional space and is defined as the expected number of other points of the process within a distance *d* of a typical point of the process divided by the intensity. This quantity can be estimated as

$$\widehat{K}(d) = \frac{1}{\widehat{\lambda} N} \sum_{i=1}^{N} \frac{\pi d^2}{A_{id}} \sum_{j=1, i \neq j}^{N} c(i, j, d),$$

Where N is the total sample size, $c(i,j,d)$ is an indicator function equal to 1 if the distance between points i and j is at most d, 0 otherwise. The quantity $\frac{\pi d^2}{A_{id}}$ is Besag's edge effect correction, A_{id} is the part of the area of the circle of radius d centered on the point i located inside the domain. Other edge correction methods include Ripley's correction, Ward and Ferrandino's correction among others and their limitations have been discussed elsewhere *(Marcon 2009).*

The *K*-function *(Ripley 1977)* was used to test consistency with or departure from spatial randomness within each site. The spatial point pattern data consisted of homesteads level geographical co-ordinates with slide positive cases and slide negative controls. The underlying heterogeneity in the population density distribution was controlled for by assessing the difference between the *K*-function summarizing the degree of clustering of locations/homesteads with cases and controls was calculated. Under the null hypothesis of no spatial dependence, the *K*-function for cases $(K_{case}(d))$ and for the controls $(K_{cont}(d))$ are identical through the distance (d). A difference in K-function $((K_{case}(d)) - (K_{cont}(d)))$ *(Diggle and Chetwynd 1991)*, also referred to the *D*-function, greater than zero suggests spatial clustering. The 95% critical regions of the observed *D*-functions for the various spatial scales examined were constructed using repeated simulations. Edge effects were corrected as described elsewhere *(Ripley 1976)*. The package spatstat in R was used to compute the D-function and the simulated envelops for statistical significance testing.

The Moran's I test evaluates local clustering or spatial autocorrelation. The Moran's I statistic is interpreted under the null hypothesis that there is random distribution of the mean age of symptomatic malaria cases. The alternative hypothesis states that spatial autocorrelation exist and can either be positive or negative. The Moran's I statistic range between -1 and 1. A positive

significant Moran's I statistic indicates a tendency towards spatial clustering, on the other hand, a negative significant Moran's I statistic indicates a tendency towards regularity (dispersion). The Moran's I statistic of 0 indicates a random distribution of events. In epidemiological studies, the Moran's I statistic is used to assess geographical similarity in continuous metrics of a disease *(Pullan et al. 2012).*

Statistical inferences on the degree of clustering can be drawn from both the Moran I and the K functions. However, these spatial functions cannot identify the position of clusters. The scan statistic on the other hand, identifies the position of local clusters but cannot be used to describe the structure of clustering and the degree of geographical auto-correlation in the same way that spatial functions do.

2.3 Statistical modelling

Fine-scale spatio-temporal variations in malaria risk has been associated with environmental and human factors *(Bannister-Tyrrell et al. 2017).* The objective of any statistical modelling framework is to determine a minimal set of covariates that sufficiently describe specific features of a given dataset *(Sauerbrei et al. 2007).* Selecting few predictors from among a set predictors is usually a challenge and can easily become arbitrary. However, covariates should be included in the model if they significantly improve model fit *(Sauerbrei et al. 2007).*

2.3.1 The linear regression model

The multiple linear regression model was used to assess linear relationships between a dependent continuous variable and a set of independent variables. Let y be a dependent variable consisting of *n* observations (y_1, …, y_n) and *p* independent variables (X_1, ..., X_p). The multiple linear regression model is given by

$$\mathbf{y}_i = \beta_0 + \sum_{j=1}^{p} \beta_j \mathbf{X}_{i,j} + \varepsilon_i$$

where, **X** - represents the p covariates in the model, the quantities $\beta_0, \ldots \beta_p$ are unknown regression coefficients/parameters; the random errors terms $\varepsilon_i | \boldsymbol{X} \sim N\ (0, \sigma^2)$.

The above specified model makes key assumptions a priori that needs to be assessed to determine their conformity. 1) linearity, 2) independent and normally distributed error terms $\varepsilon_i | \boldsymbol{X} \sim N\ (0, \sigma^2)$ and 3) Constancy of residual variance. However, these assumptions are not usually realistic and in most cases violated. Variable transformation to linearize nonlinear relationship between the response and covariate(s) are recommended and these would include polynomials and the cubic splines just to mention a few.

Coefficient of determination (R^2)

R^2 is the ratio of the total empiric variance explained by the model to the total empiric variance of the response variable. The adjusted R-squared coefficient (R_a^2) is defined to be:

$$R_a^2 = 1 - \frac{N-1}{N-p-1}(1 - R^2)$$

where p denotes the number of independent variable included in the model. The component $(\frac{N-1}{N-p-1})$ penalizes for the model complexity.

2.3.2 The logistic regression model

The logistic regression model was used to assess the heterogeneity in trends by comparing a model with and without space-time interaction effects using the likelihood ratio test statistic. The model was formulated as follows:

$$logit(p_i) = \ln\left(\frac{p_i}{1-p_i}\right) = \beta_0 + \sum_{j=1}^{m} \beta_j \mathbf{X}_{i,j}$$

where p_i is the probability of a slide positive for patient i, **X,** represents the covariates (time and the dummy variables generated from location and location-time interaction) while $\beta_0, \ldots, \beta_m$ are the regression coefficients indicating the relative effect of the explanatory variable on the outcome (m is the number of continuous and dummy variables generated from the categorical covariates in the model).

The relationship between the binary response variable and the covariates was assessed using multiple logistic regression model. To select a parsimonious final model, covariates were added to the model in a forward stepwise fashion and the Likelihood Ratio Test (LRT) used to compare nested models. This procedure identified a set of covariates which resulted in a significant reduction in $-2Log\hat{L}$ at 5% significance level.

The likelihood ratio test statistic was computed as follows:

$$Likelihood\ ratio\ statistic\ (D) = \left(-2ln \frac{likelihood\ of\ the\ reduced\ model}{likelihood\ of\ the\ Complex\ model}\right),$$

The degrees of freedom are equivalent to the difference between the number of parameter estimates between the more complex model and the reduced model.

Potential multicollinearity was assessed by examining correlations between continuous predictor variables and formally by using the variance inflation factor. The effect of collinearity was mitigated by selecting covariates that were most significant in the model. The variability accounted by the model in the logistic regression analysis was measures using the psedo-R^2.

Pseudo-R^2

In linear regression models, the coefficient of multiple determination (R^2) measures the amount of variation in the outcome variable explained by the covariates. Generalizing R^2 to logistic

regression models has proved difficult. Various measures have been proposed in literature and their limitations discussed *(Mittlböck and Schemper 1996)*. The Pseudo-R^2 also referred to the McFadden's likelihood ratio index is defined as:

$$R_{McFadden} = 1 - \frac{l_c}{l_{null}}$$

where l_c and l_{null} are the maximized log-likelihood of the complex model and the null model respectively. A criticism to McFadden's index is that when applied to linear regression model, it is not equivalent to the coefficient of multiple determination thus creating an interpretation challenge as a measure of explained variation in logistic models. However, despite the drawbacks, McFadden's index can be used to give some sense of how much variation in the outcome is being explained by the predictors, and is the most widely accepted measure for logistic regression.

Presence of clusters within the study population should be accounted for during statistical analysis. Ignoring clustering may result to inflated significance, that is, smaller p-values and narrower confidence intervals than would be expected if clusters were absent *(Altman and Bland 1997)*. Robust standard errors for predictors of malaria in Kilifi County were sort when clustering was suspected using models accounting for clusters. Sources of clustering of malaria transmission could result from unmeasured predictors that could range from genetic factors at homestead level, quality of housing, proximity to water bodies among others.

2.3.3 Model-based geostatistics

Geostatistical modelling is an extension of standard regression modelling to account for spatial/temporal autocorrelation. Geostatistical models are frequently used in malaria epidemiology to generate disease maps that can subsequently be used to guide interventions

strategies. These models take advantage of spatial and temporal autocorrelation ("borrow strength" from neighboring homesteads/villages) to interpolate missing data and hence producing smooth maps *(Giardina et al. 2012).* The models are reliable when spatial autocorrelation is evident and covariate information do not fully account for spatial patterns in the dataset and thus the need to incorporate spatial dependencies in the modeling framework. Health monitoring and evaluation teams have utilized these models to produce malaria risk maps in Africa *(Noor et al. 2014),* Senegal *(Giardina et al. 2012),* Zambia *(Riedel et al. 2010),* Zimbabwe *(Mabaso et al. 2006),* Ghana *(Kasasa et al. 2013),* Kenya *(Stresman et al. 2017)* just to mention a few, and in parasitic infections *(Soares Magalhaes et al. 2011, Karagiannis-Voules et al. 2013, Scholte et al. 2014)* and other diseases *(Wimberly et al. 2013).* The models were however not applied in this thesis, because my objectives were to describe and examine hotspots in directly measured datasets rather than to impute data that was not directly measured.

2.4 Multiple fractional polynomial (MFP) transformations

The multiple fractional polynomial algorithm was used as previously described *(Sauerbrei and Royston 1999, Sauerbrei et al. 2006)*. MFP model selection begins with a model of predefined complexity and uses a backward elimination procedure to simplify it. This procedure preserves the familywise error rate.

To illustrate the procedure a model with a single continuous covariate (x) is used for simplicity. The linear model is defined by $\beta_0 + \beta_1 x$ and in some instances, provides an adequate description of the response-covariate relationship. However, in most instances the assumption of linearity if often violated and thus nonlinear models need to be investigated to improve the model fit. The fractional polynomial transformation is a feasible choice. The first-degree fractional polynomial (FP1) function is formulated as follows: $\beta_0 + \beta_1 x^p$. The power "p" is an element of a restricted

set S = (−2, −1, −0.5, 0, 0.5, 1, 2, 3), where x^0 denotes a log transformation. FP1 functions can be extended to the more complex and flexible two-term FP2 functions with powers (p1, p2) defined as $\beta_0 + \beta_1 x^{p1} + \beta_1 x^{p2}$ with p1 and p2 taken from the set S. If p1 = p2, the proposed equation is $\beta_0 + \beta_1 x^{p1} + \beta_1 x^{p1} * \log x$, referred to as the repeated-powers FP2 model. Given the restricted set of powers for "S", there are fundamentally 8 FP1 transformations, $\binom{8}{2} = 28$ FP2 transformations with distinct powers, and 8 FP2 transformations with equal powers (p1 = p2) *(Sauerbrei et al. 2007).*

To enhance parsimony and the stability of the model during function selection a sensible default function is required. In this case a linear function is a natural choice. It follows that unless the data supports a more complex FP function, a straight line is preferred. The suitable model is selected in 3 steps: 1) a comparison between the best FP2 model with the null model is made based on a likelihood ratio test, 2) If significant, FP2 model is tested against a straight-line model, 3) if also significant, a final step compares the best FP2 and FP1 models as described in detail elsewhere *(Royston and Sauerbrei 2003).* MFP transformations were used to allow for nonlinear effects of the covariates in the regression models.

2.5 Meta-analysis

Meta-analysis is a quantitative statistical analysis tool that combines findings from several similar but independent studies or trials to test statistical significance for a pooled estimate. The aim is to increase power and precision of the estimates *(Mulrow 1994).* This method, implemented in the Stata package metan *(Harris et al. 2008),* was used to pool correlations from multiple studies conducted within sub-Saharan Africa. Individual estimates and the pooled estimate were graphically presented using forest plots *(Harris et al. 2008).* Heterogeneity was

assessed by visual inspection of the forest plots to detect overlapping CI and statistically by using an I^2 test statistic in which values circa 50% indicate moderate heterogeneity. Fixed effect meta-analysis was used except when the I^2>50%, in which a random effect meta-analysis was applied. Stata's user-written function (metaninf) *(Steichen 2001)* was used to conduct sensitivity analyses to examine the influence of each study site on the pooled correlation estimate. This was examined by omitting one study at a time and then assessing the influence of the omitted site, that is, a site was considered influential if the pooled correlation estimate without that site was not within the 95%CIs of the overall pooled correlation.

Funnel plots *(Sterne et al. 2001),* which are plots of effect estimates against sample size were used to assess publication bias. The funnel plot is built on the premise that the precision of the effect size increases with increasing sample size. In the absence of bias, the plot resembles a symmetrical inverted funnel. Otherwise, skewed asymmetrical funnel plots indicates bias *(Egger et al. 1997).* Stata function metafunnel was used to graph funnel plots for assessing reporting bias in meta-analyses.

2.6 Multiple imputation.

Missing data occur in most epidemiological and clinical research and is mostly unavoidable due to nonresponse and subject attrition *(Rubin 2004).* For instance, mortality before taking measurement at a hospital setting or completing follow-ups introduces missing data that inherently reduce the power with which parameters are estimated thus potentially undermining the validity of research conclusions. Interest towards addressing missingness during data analysis has been on the increase. Multiple imputation can potentially reduce bias, increase efficiency (reduce standard errors) and is now available in standard statistical softwares *(Horton and Lipsitz 2001).* Unlike the classical complete case analysis, where only complete data points

are included in the analysis, multiple imputation ensures that data points that are missing are filled and a full dataset can be used for the final analysis.

In the presence of missing data, the validity of multiple imputation analysis depends on the type of missingness and the variables included in the imputation model *(Sterne et al. 2009)*. There are three types of missingness: 1) missing completely at random (MCAR)- in this case there is no systematic differences between the missing data and the observed ones, 2) missing at random (MAR) occurs when systematic differences between missing values and the observed ones can be explained by differences in the observed data, and 3) missing not at random (MNAR) occurs when systematic differences between missing values and the observed ones are not fully explained by differences in the observed data *(White et al. 2011)*. Complete case analysis is valid if the likelihood of being a complete case given the covariates in the model, is independent of the outcome. Multiple imputation approaches are valid under the assumption of MAR. However, when factors associated with both the outcome and missingness are not included in the imputation model (MNAR), multiple imputation will not fully remove bias. To detect the mechanism of missingness, careful preliminary analysis to identify the scope for multiple imputation and to provide guidance for building the imputation model is required *(Sterne et al. 2009)*.

In epidemiological settings, it is possible for missing values to occur in more than one variable. Multiple imputation with chained equations (MICE) allows a set of multiple imputation models, one for each variable with missing data to be applied concurrently *(White et al. 2011)*. MICE can handle different variable types including; continuous, binary, ordered and unordered categorical variables. Three to five imputed datasets are theoretically sufficient *(Allison 2000)*, however, in practice more imputed datasets are recommended *(Horton and Lipsitz 2001, Graham et al. 2007)*. Analysis of each imputed dataset is done separately and the parameter

estimates combined using Rubin's rules *(Rubin 2004)*. That is, suppose $\hat{\theta}_j$ is the regression coefficient of interest from a regression model obtained from the j-th imputed dataset. The respective post-imputation combined estimate is the average of individual estimates:

$$\bar{\theta} = \sum_{j=1}^{k} \hat{\theta}_j / k$$

where $\hat{\theta}_j$ is the estimate of θ using the j-th imputed dataset and k is the number of imputed datasets. The associated standard error is a combination of the within and between-imputation variances *(White et al. 2011)* formulated as follows:

$$SE(\bar{\theta}) = \sqrt{\frac{\sum_{j=1}^{k} var(\hat{\theta}_j)}{k} + \left(\frac{k+1}{k}\right) * \frac{\sum_{j=1}^{k} (\hat{\theta}_j - \bar{\theta})^2}{k-1}}$$

For a sufficiently large number of imputed datasets, the combined estimate follows an approximate Gaussian distribution.

Preliminary analysis to assess the mechanism of missingness was done prior to multiple imputation. This involved assessing the association between missing data with covariates and the outcome. MICE was conducted in STATA software as indicated in chapter 3. 50 imputed datasets were used to impute missing data on personal ITN use. In the imputation model, the outcome variable and the covariates were included as previously recommended *(Sterne et al. 2009)*. Standard errors were calculated using Rubin's rules accounting for variability in results among imputed datasets. Results from complete case analysis and multiple imputation were reported and compared.

The above statistical methods were used in the analyses presented in chapter 3, 4 and 5. Hereafter I will mention the methods used without giving details but with reference to this

chapter. Any additional statistical methodology specific to any given chapter will be discussed in the relevant chapter.

Chapter Three

3 Age, spatial and temporal variations in hospital admissions with malaria in Kilifi county

3.1 Introduction

Marked declines in malaria transmission intensity and related mortality has been witnessed in parts of sub-Saharan Africa *(Ceesay et al. 2010, O'Meara et al. 2010, Noor et al. 2014)*. It is known that children acquire immunity to malaria following repeated exposure and there is an inverse relationship between the intensity of malaria transmission and age of susceptibility to malaria *(Snow et al. 1997, Woolhouse 1998)*. The shift in age of susceptibility to malaria following implementation of malaria control interventions has been reported in both field conditions *(Okiro et al. 2009, Trape et al. 2011, Karema et al. 2012)* and predicted by simulation studies incorporating acquired immunity *(Pemberton-Ross et al. 2015)*.

3.2 Rationale

In areas of high transmission intensity, younger children present to hospital with severe anaemia as the main complication of *P. falciparum* infection. In areas with less intense transmission, cerebral malaria predominates slightly older children with relatively higher mortality rate. It is therefore essential to monitor outcomes following initial reductions in malaria transmission.

In this chapter, I present data from a 25-year longitudinal surveillance of hospital admission with malaria to Kilifi county hospital on the Kenyan coast.

3.3 Study objectives

1. Describe trends of malaria admissions by age group over the 25-year period
2. Assess the spatial and temporal heterogeneity of malaria transmission in Kilifi county

3. Assess the effectiveness of personal and community ITN use
4. Examine the effect of EVI on malaria transmission as a proxy for soil moisture content which may correlate with the presence of mosquito breeding sites.

3.4 Methods

3.4.1 Kilifi county and population

Kilifi County is located along the Kenyan coast and to the north of Mombasa. The county has a population of approximately 1.11 million people *(KNBS 2009)* and covers an area of approximately 12,245.90 km^2. The region is predominantly rural, with less than 15% of the population living in the urban area of Kilifi town *(Scott et al. 2012)*. This region has been endemic for malaria transmission, though in the recent past declining transmission has been witnessed *(O'Meara et al. 2008, Noor et al. 2009a)*. Kilifi District Hospital (KDH) recently renamed Kilifi County Hospital (KCH) and the Kilifi Health and Demographic Surveillance System (KHDSS) are part of Kilifi County (Figure 3.1) and play a major role in providing healthcare services. Similarly, the Kenya Medical Research Institute (KEMRI) and KEMRI-Wellcome Trust Research Programme is based at the hospital and primarily conduct research on malaria and other diseases *(Scott et al. 2012)*. The population around Kilifi mainly depends on subsistence farming, tourism and fishing as the major economic activities.

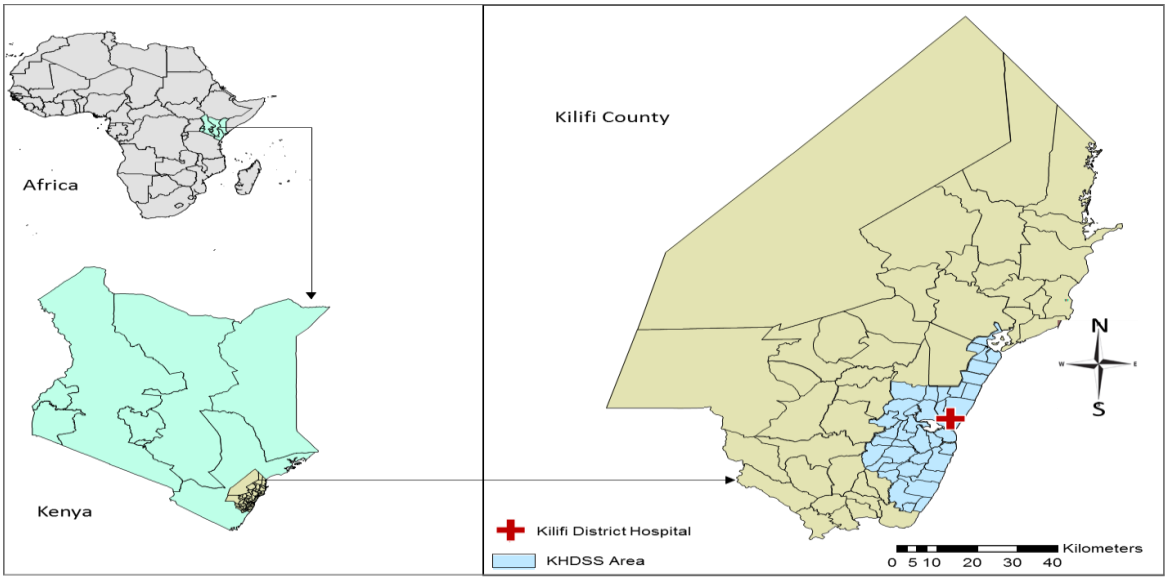

Figure 3.1*: Map of Kilifi county indicating the hospital and the KHDSS area*

The main crops are maize and tree crops such as coconuts and cashew nuts. Low soil fertility coupled with low unreliable rainfall over the years has led to persistent poor crop yield making the county one of the poorest in Kenya. The long rains are expected between April and July and the short rains between November and December, but considerable year to year variation in the amount of rainfall has been witnessed.

3.4.2 The KHDSS

The study area and population characteristics have been described in detail elsewhere *(Scott et al. 2012)*. Briefly, the KHDSS (http://www.kemri-wellcome.org/khdss) was established in the year 2000, to provide a platform upon which hospital and community based studies conducted at the site recruit and locates study participants. The area has been mapped using global positioning system (GPS) and digital maps produced. Field work personnel conduct re-enumeration and vital status registration to update the population register by visiting every homestead three times a year. KHDSS covers an area of about 891km^2 and has a residence

population of about 260,000 (March 2011). Mapping and census are done and recorded into a population register linked to inpatient records through an integrated data management system. The KHDSS population register is constantly updated for births, deaths and in- and out-migration events every 4 months. Approximately 70% of the children presenting for admission at the Kilifi County Hospital are linked to their residence information using the system while the rest are mostly non DSS residents.

3.4.3 Malaria control in the KHDSS area

The Kilifi Health and Demographic Surveillance (KHDSS) area has seen several malaria control programs even before its inception in 2000. For instance, the first ITN study was conducted between July 1993 and July 1995 in the northern part of Kilifi and demonstrated that ITN use provides personal protection against malaria *(Nevill et al. 1996)*. In 2002, Population Service International (PSI) launched a retail campaign for ITN distribution *(Noor et al. 2006)*. This campaign was boosted by the delivery of highly subsidized ITNs through Maternal and Child Health (MCH) clinics towards the end of 2004 *(Okiro et al. 2007)*. In September 2006, the Ministry of Health launched a campaign of free distribution of ITNs to children under the age of five years *(Noor et al. 2007)*. This campaign was repeated later in 2013 with the motive of increasing ITN coverage. Besides the various campaigns on ITN use, other control measures have been rolled out that include the government policy changes on 1st line drug treatment of malaria in government clinics. That is, before the year 2000, chloroquine was used as the 1st line treatment against malaria *(Shretta et al. 2000)* despite reports of drug resistance and increased human to vector transmission *(Hogh et al. 1998)*. It was due to the overwhelming evidence against chloroquine that a policy changeover to sulphadoxine-pyrimethamine (SP) was effected in mid-2000 lasting to around April 2006 when another policy changeover was effected to

artemether-lumefathrine (AL) *(Shretta et al. 2000).* Another control method seen in this study area involved training shopkeepers on treatment compliance in Chonyi area. These control methods may have contributed in suppressing the incidence of clinical malaria in some parts of the study area *(Marsh et al. 1999).* The trend in malaria parasite prevalence on the Kenyan coast and the combination of factors which may explain the changes observed between 1974 and 2014 is summarized in Figure 3.2 below.

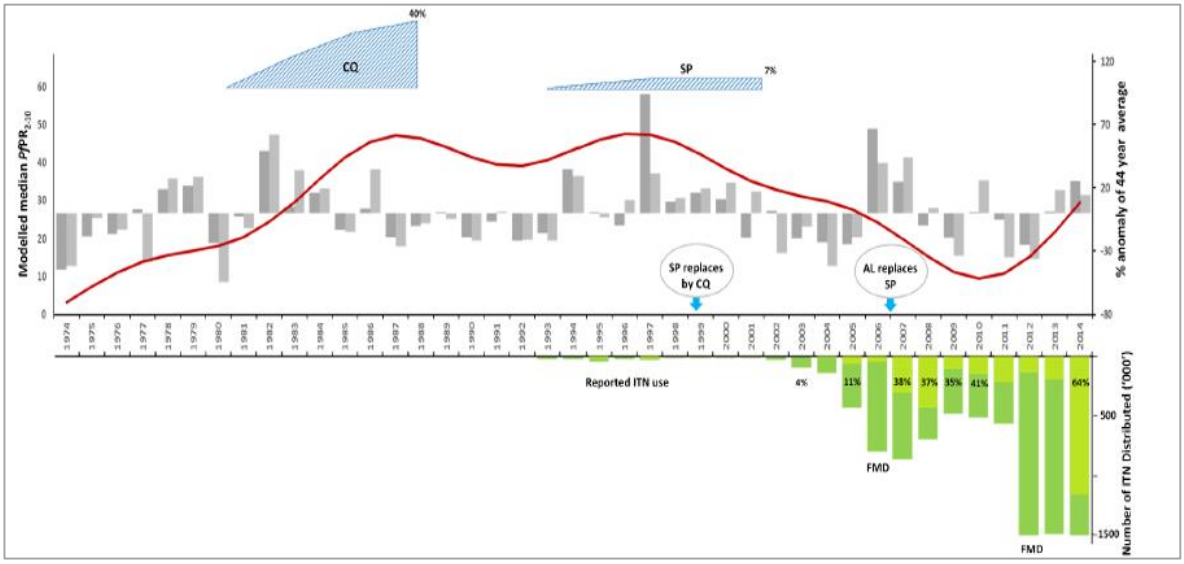

Figure 3.2 ***Prevalence of malaria on the Kenyan coast and the combination of factors which may explain the changes since 1974.***

Extracted monthly GAM fitted median Pf$PR_{2\text{-}10}$ *(red line) shown in relation to annual and long rains (March-June) percentage anomalies in precipitation (dark and light grey bars respectively); cumulative "effective" mid-year ITN distribution data (dark green likely efficacious, light green <50% efficacious); estimated day 7 anti-malarial drug failures to clear parasitaemia based on information provided in text (blue triangles); and malaria policy milestones for standard treatment guidelines and mass ITN distribution dates (FMD), including reported ITN use among all age groups(Snow et al. 2015).*

3.4.4 Kilifi county hospital surveillance system

KCH provides primary care services and acts as the only referral facility within the KHDSS area. Upon admission, KHDSS residents received a unique personal identifier that links them to their demographic details. Between 1989 and 2002, admitted patient's residence were known to sublocation level after which homestead level GPS coordinates were available. Laboratory records and standard clinical data for all admissions were linked to demographic records for the residents of KHDSS area using personal identifiers. For most residents of the KHDSS, KCH is the nearest facility offering inpatient care, however, two more facilities offering similar inpatient service are the Malindi District Hospital and the Coast Provincial General Hospital that are within 20 kilometers to the North and South of the KHDSS respectively.

Screening for malaria parasites at the KCH paediatric admission wards has been continuous since the establishment of a pediatric admission ward surveillance system in 1989. These assessments are conducted on all emergency admissions, irrespective of presumptive diagnosis, except for those admitted for elective surgery.

3.4.5 Ethical consideration

Approval for human participation in the hospital surveillance and the cohorts was given by the KEMRI Scientific Steering Committee and the Ethical Review Committee of KEMRI. The studies were conducted per the principles of the declaration of Helsinki.

3.4.6 Data collection

The study was conducted at KCH situated at the center of the KHDSS area as described above. Children <13 years of age were admitted to the pediatric service, where demographic details and clinical history were recorded and blood samples were collected and examined for malaria parasites by microscopy. Children with signs of severe disease such as impaired consciousness

or deep breathing were assigned to the high dependency unit, whilst children without such signs were admitted to the pediatric ward. Research clinicians provided 24-hour clinical cover of both the high dependency unit and the general pediatric ward.

Thick and thin blood smears were stained with 10% Giemsa and examined at x1000 magnification for *P. falciparum* malaria parasites. One hundred microscopy fields were examined before slides could be considered negative. Microscopy standards were monitored through a quality assurance scheme including training on induction and at regular intervals for microscopists and the use of external quality control slides.

3.4.7 Data analysis

The analysis included children admitted between 1990 and 2014 to KCH, aged between 3 months and 13 years, and residing within the KHDSS area. Key metrics were the Malaria Positive Fraction (MPF) and the mean age of the children admitted with positive malaria slides (i.e. without applying a threshold parasitaemia). The MPF describes the fraction of acute admissions to hospital with positive blood films compared with all admissions and is also known as the "Slide Positive Rate" *(Jensen et al. 2009)*. MPFs from passive surveillance at the hospital were compared with incidence data from active surveillance of 2 cohorts in the community, conducted as previously described *(Mwangi et al. 2005, Bejon et al. 2007)*. Unpaired t-test were used to test the difference in means when variables were normally distributed, otherwise the Wilcoxon rank-sum test was used to compare average/median age between groups. Multiple fractional polynomials (MFPs) were used to assess nonlinear associations in regression models. A sensitivity analysis was carried out with a "malaria case" defined using a cut-off parasitaemia of >2,500 parasites per µl.

3.4.8 Spatio-temporal models

The spatio-temporal heterogeneity of malaria transmission in Kilifi County was assessed using a multivariable logistic regression model (refer to chapter 2 for details on the methodology) with the presence or absence of malaria parasites by microscopy among medical admissions (excluding trauma and elective surgery) as the outcome variable, time (continuous variable to capture the secular trends), year (categorical to capture year-to-year variability), region (referring to North vs South of the creek), location (referring to administrative areas within region) and space-time interactions as the independent covariates. I used the Pseudo R^2 to assess the contribution of the various components of the model. As a measure for the background community prevalence of parasitaemia, age-standardized parasite prevalence estimates among trauma cases were determined using standard methods *(Smith et al. 2007a)*, but only when children were afebrile on admission and clinical assessment did not reveal any other acute cause for admission.

3.4.9 Predictor variables

Enhanced Vegetation Index (EVI) data between 2000 and 2014 were used as a proxy for soil moisture*(Midekisa et al. 2012)*. EVI data were downloaded at a 250x250m^2 pixel resolution for 16 day intervals. I extracted the EVI bands using ArcGIS and saved them in the georeferenced tagged image file format (GeoTIFF). The GeoTIFF files were then processed into data using QGIS batch processing functionality and scaled to between 0 and 1. The EVI dataset was aggregated to a year.

Data on ITN use were obtained from yearly KHDSS surveys targeting all residents between 2009 and 2014. I used data from responses to the question “did you sleep under an ITN last night?” or “did your child sleep under an ITN last night?”

Regular geographical grids were imposed on the study area and concentric circles were generated around each admitted child's residence using longitude and latitude coordinates. The prevalence of ITN use within each concentric circle and the mean EVI within each grid space was used in multivariable models. Model parsimony from the resulting sets of EVI and ITN covariates created was determined using a forward stepwise selection criterion and the likelihood ratio testing done to formally compare models. The superiority of non-linear models over linear models was assessed visually by plotting residuals against covariates, and statistically by testing the significance of multiple fractional polynomials over linear fits where asymmetrical distributions of residuals were identified as described in chapter 3. Observations with missing locations, age and slide result were excluded prior to the analysis. Multiple imputation with 50 imputed datasets was used to impute missing data on personal ITN use. The variables community ITN use, malaria positivity, EVI, and age were used in the imputation model. The imputed datasets were combined as per Rubin's rules described in chapter 2. Age standardized parasite prevalence was computed in R (version 3.0.2) and all other analysis were done using Stata 12.0 (Stata Corp., College Station, TX, USA).

3.5 Results

Over 25 years, there were 107,218 pediatric admissions recorded, of whom 87,909 were known residents of the KHDSS and 71,438 were ≥3 months or <13 years of age. Malaria slide data were missing for 2,334 leaving 69,104 for our analysis (Figure 3.3). The characteristic of the study population is presented in Table 3.1.

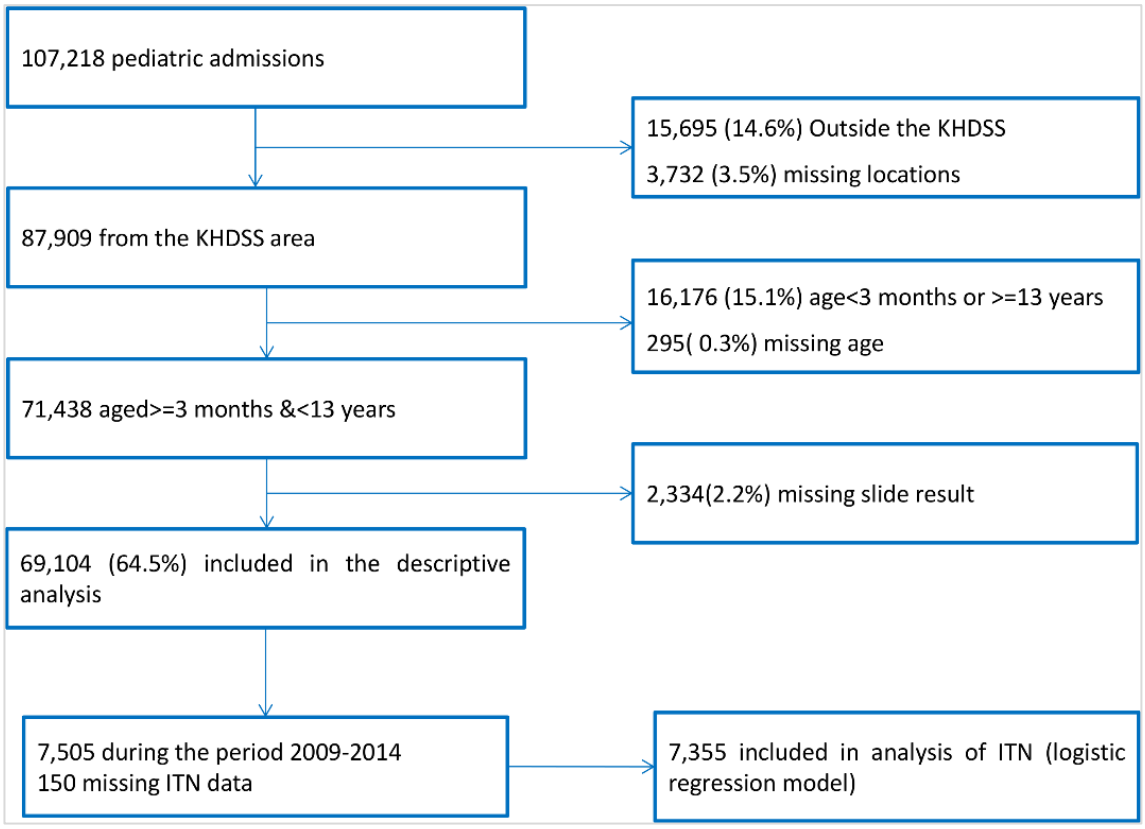

Figure 3.3*: Flow diagram of participant numbers. Participant numbers and reasons for exclusion at each stage are shown.*

Table 3.1*: Characteristic of the study population*

Period in which data was available	Analysis Type	Characteristics	Malaria Slide Positive	Malaria Slide Negative
		Admissions N(%)	26883(38.9)	42221(61.1)
		Age (months) median(IQR)	25.9(13.2-44.9)	19.2(9.7-42.7)
		Male, No. (%)	14,217(53.0)	23,862(56.6)
		Locations		
	Descriptive Analysis	Chonyi No. (%)	2,774 (10.3)	3,955 (9.4)
		Gede No. (%)	695 (2.6)	1,856 (4.4)
1990 to 2014		Jaribuni No. (%)	2,018 (7.5)	2,383 (5.7)
		Junju No. (%)	2,018 (7.5)	3,038 (7.2)
		Mtwapa No. (%)	1,182 (4.4)	3,160 (7.5)
		Ngerenya No. (%)	1,246 (4.6)	1,941 (4.6)
		Roka No. (%)	1,964 (7.3)	2,960 (7.0)
		Sokoke No. (%)	1,470 (5.5)	1,861 (4.4)
		Takaungu No. (%)	5,071 (18.9)	4,590 (10.9)
		Tezo No. (%)	8,404 (31.3)	16,437 (39.0)
2009 to 2014		Admissions N (%)	1294 (17.2)	6,211(82.8)
	Logistic regression Model	Age (months) median(IQR)	48.1 (30.1- 73.1)	24.2 (11.8-54.3)
		Missing Personal ITN use N(%)	47(3.6)	103(1.7)
		Missing data on ITN during Community Surveys N(%)	190,417 (12.8%)	

3.5.1 Access to care

I first tested for evidence of a bias in MPF by access to care. In order to assess the effect of access to care, I examined the data for trends in MPF over the euclidean distance between children's residence and the hospital (Figure 3.4A). Best-fit lines showed greater heterogeneity in MPF over distance than linear trend in MPF over distance (a non-significant gradient of -0.003 per km, 95%CI -0.006 to 0.0003, p=0.08), suggesting that geographical heterogeneity had a greater effect than any bias that may be introduced by distance from the hospital. Furthermore, I found that the incidence of malaria on active case detection from 2 cohorts monitored over 10

years *(Mwangi et al. 2005, Bejon et al. 2007)* correlated closely with MPF measured at the hospital (r=0.84, p<0.001, Figure 3.4B).

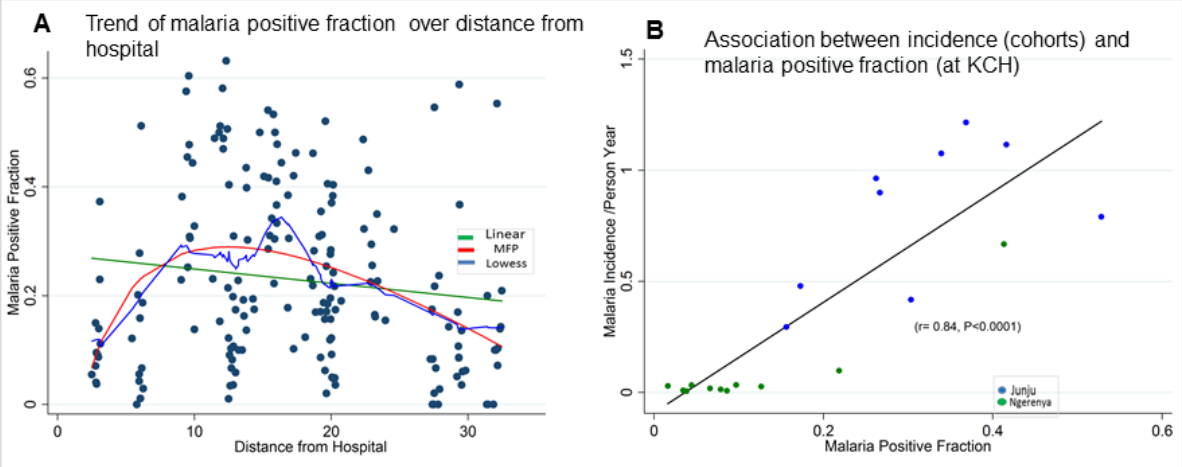

Figure 3.4*: Association between MPF, distance from hospital and malaria incidence.*

Panel A shows the association of Malaria Positive Fraction with Distance from Hospital (km), the green line presents the predicted linear regression line, the red line presents the predicted line from the multiple fractional polynomial model and the blue line presents the fitted lowess function. Panel B shows an association between malaria incidence from active case detection in Junju and Ngerenya with the MPF among patients admitted in hospital from these sites.

3.5.2 Geographical distribution and trends in malaria positive fraction

The fraction of acute admissions that were positive for malaria (i.e. the "Malaria Positive Fraction" or MPF) for the full dataset was 0.389 (95%CI 0.385 to 0.393). MPF was subject to marked spatial and temporal heterogeneity (Figure 3.5A). There was a decline in MPF over the years with the lowest MPF (i.e. 0.07 (198/2858)) being recorded in 2009, and a subsequent increase in MPF (up to 0.24 (511/2169)) in 2014 (Figure 3.5A). I therefore refer to the 1990-2002, 2003-2008 and 2009-2014 as "pre-decline", "decline" and "post-decline" periods, respectively.

The average age of children admitted with malaria positive slides and acute illness increased gradually from 20.2 months (95%CI 18.9 to 21.6) in 1990 to 45.3 months (95%CI 42.5 to 48.3) in 2014 ($p<0.001$). During the same period the mean age for children with malaria negative slides and acute illness increased only slightly from 19.8 months (95%CI 18.6 to 21.1) in 1990 to 26.1 months (95%CI 24.9 to 27.5) $p<0.001$ in 2014 (Figure 3.5B). Furthermore, the mean age of children with asymptomatic parasitaemia among trauma admissions did not increase significantly ranging from a geometric mean of 52.1 months (95%CI 49.5 to54.9) in the 1990-2002 pre-decline period to 64.6 months of age, 95%CI 49.5 to 84.2 in the 2009-2014 post-decline period, $p= 0.0543$ (the latter wide confidence interval reflecting the reduced prevalence of asymptomatic infection).

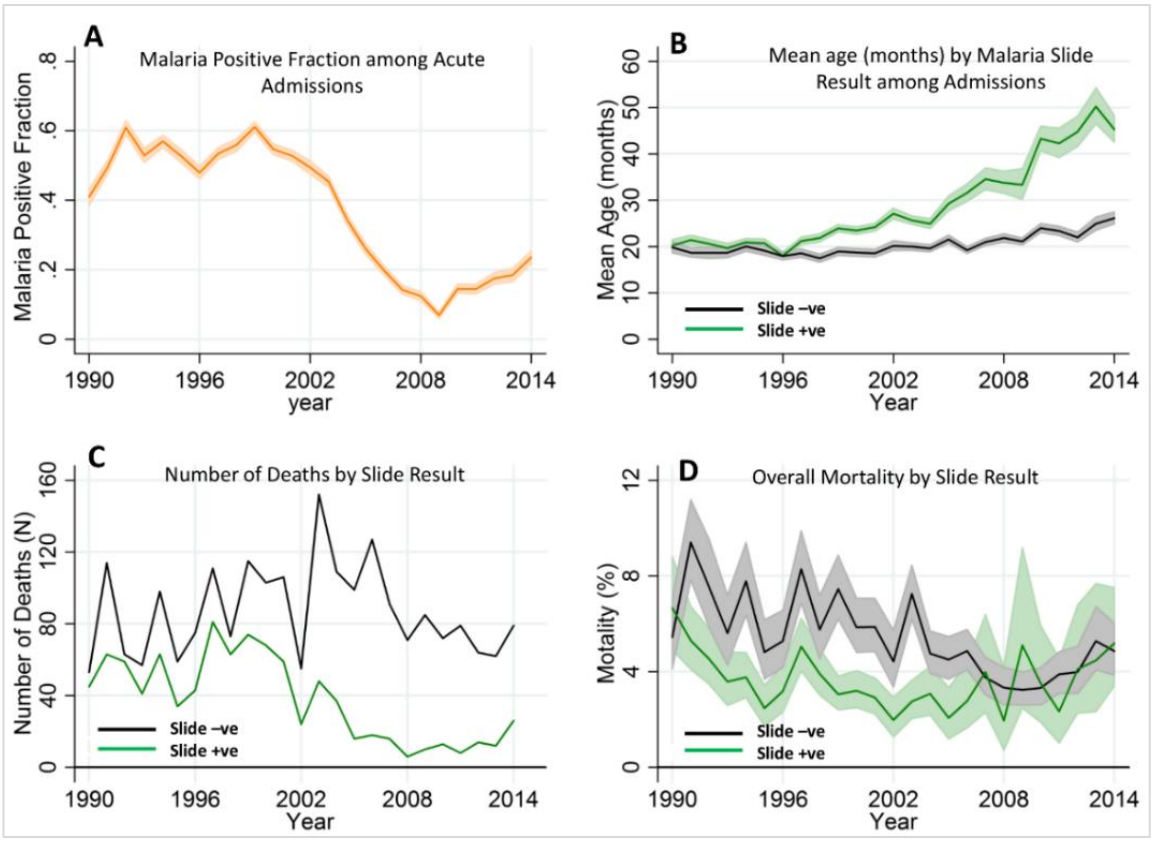

Figure 3.5*: Temporal trends of malaria positive fraction, age of slide positive and mortality among acute admissions.*

Panel A shows the temporal trend of Malaria Positive Fraction (MPF). Panel B shows the trends of mean age over time for the slide positive and slide negative admissions. Panel C shows the temporal trends of absolute number of deaths and Panel D shows case fatality rates.

The absolute number of malaria slide positive deaths recorded in the hospital surveillance fell over time, although the absolute number of malaria slide negative deaths did not show a clear trend (Figure 3.5C). The case fatality rate (i.e. the proportion of children admitted who died) among malaria slide positive children was static, although among slide negative children the case fatality rate showed a gradual decline (Figure 3.5D).

Models accounted for variation in MPF poorly in the pre-decline period, however the decline period was dominated by temporal variation, and the post-decline period was dominated by spatial variation (Figures 3.6A, 3.6B and 3.6C, respectively).

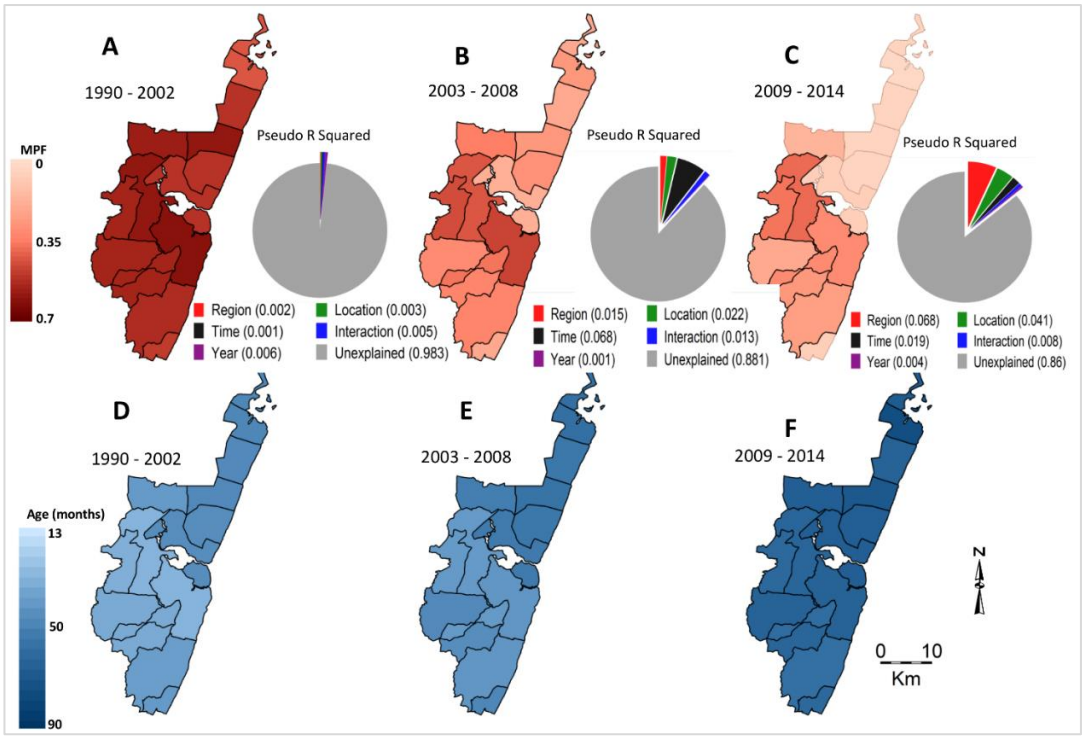

Figure 3.6*: Geographical distribution of malaria positive fraction and age of slide positive acute admissions over time.*

Panels A, B and C show spatial distributions of MPF for the Pre-decline, Decline and Post-decline periods respectively, and their associated pie charts show the proportion of the variability in MPF explained by the predictors; Region (i.e. North vs South), Location, Time trend (i.e. as a continuous variable), an interaction between time and location, year to year variation (i.e. year as a stratified variable) and the unexplained variations. Panels D, E and F show spatial distributions of age in months for the slide positive admissions during the Pre-decline, Decline and Post-decline periods.

There was an inverse relationship between MPF and average age of children with positive slides when averaged by location: areas with higher average age of malaria tended to have lower MPF and vice versa (Figure 3.6A compared to 3.6D, 3.6B compared to 3.6E and 3.6C compared to 3.6F). This was statistically significant before the decline ($r= -0.36$, $p<0.001$), but not after ($r= -0.05$, $p=0.66$). There was a shift in the burden of disease from younger age groups to older age groups after the decline (Figure 3.7).

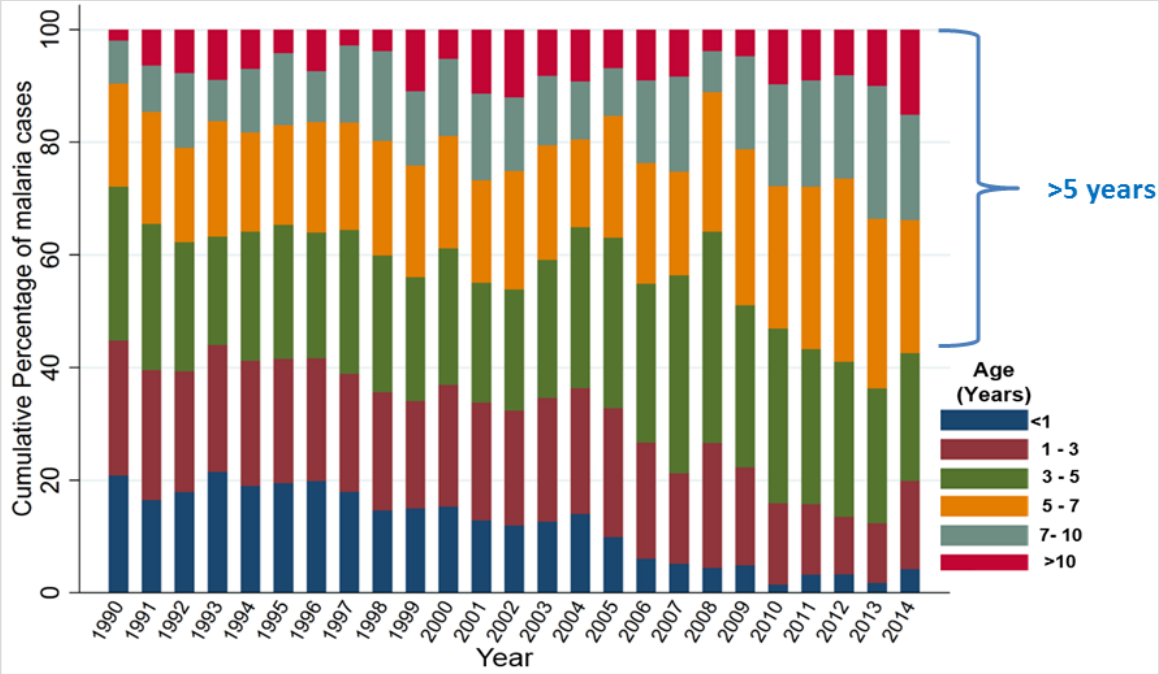

Figure 3.7*: Trends of malaria positive fraction by age group (years).*

The graph show the trend of malaria positive fraction in each age category <1 (less one year old), 1-3 (1 to 3-year-old), 3-5 (3 to 4-year-old), 5-7 (5 to 7-year-old), 7-10 (7 to 10-year-old), >10 (children over 10-year-old).

3.5.3 Is the post-decline increase in malaria due to falling immunity?

In order to disentangle the effects of loss of immunity from transmission intensity, I hypothesized that MPF for children <1 year old ($MPF_{<1yr}$) would indicate transmission intensity without the offsetting of acquired immunity (as has been done previously *(Snow et al. 1996, Bejon et al. 2014)*). I examined the trends in $MPF_{<1yr}$ by region (i.e. using the Creek in the center of the County as the point of division into Northern vs Southern regions) in view of the significant effect of region on variability of MPF (Figure 3.6C). $MPF_{<1yr}$ showed a clear decline from the mid-1990s down to almost zero in the Northern region by 2008 and to low levels in the Southern region from 2008 onwards, with a very slight increase after 2009 in the North and a slightly more marked but variable increase in the South after 2009 (Figure 3.8A). In contrast,

$MPF_{>1yr}$ showed a greater increase in both regions in the post-decline period, which was particularly marked in the South (Figure 3.8B). Within geographical locations, malaria positivity showed substantial variability from year to year (Figure 3.8C).

I also examined the age-corrected parasite prevalence ($PfPR_{2-10}$) among the (3,971/69,104) 5.8% trauma admissions (excluding trauma cases with fever) as a proxy of community parasite prevalence (Figure 3.8D). $PfPR_{2-10}$ which declined from (30/72) 43% and (35/62) 56% in the Northern and Southern regions, respectively in 1999, to <1% (i.e 0/151 in the North and 1/100 in the South) in both regions in 2009, but subsequently rose, reaching (2/102) 2% and (3/83) 4% in the Northern and Southern regions, respectively, in 2014.

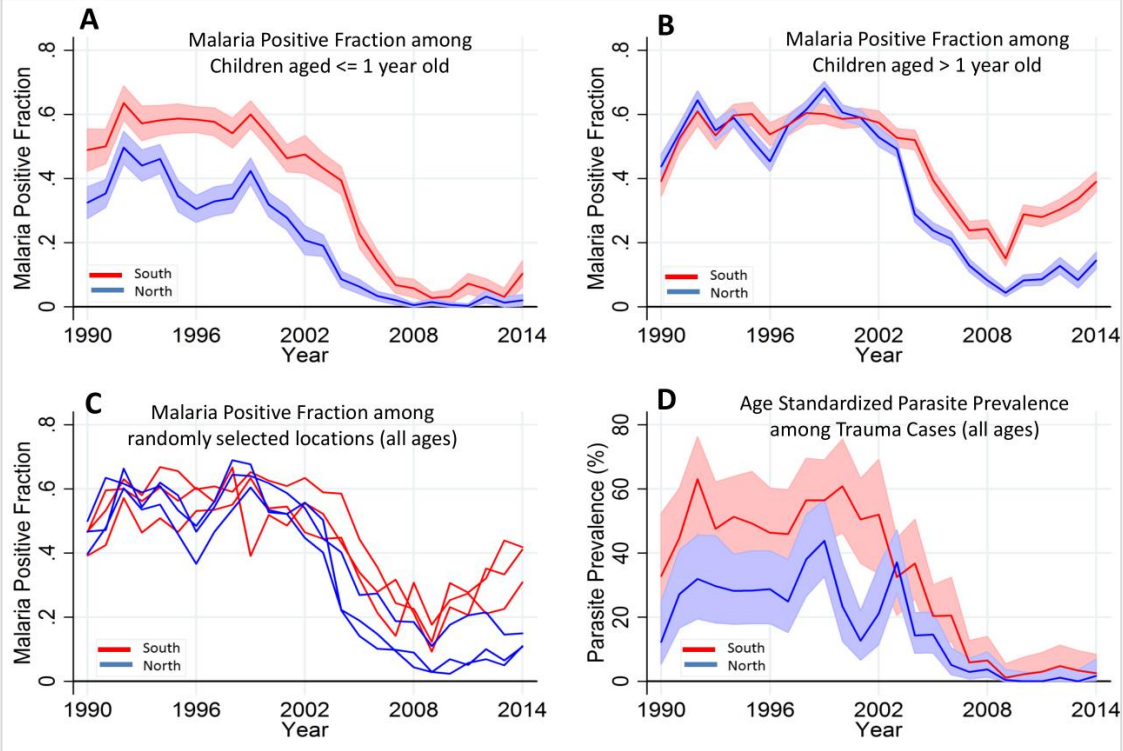

Figure 3.8*: Temporal trends of malaria positive fraction by age and parasite prevalence.*

Panels A and B show the temporal trends of MPF in admitted children aged<=1-year-old and children aged>1-year-old respectively, red line represents the Southern region while the blue line represents the Northern region of the hospital. Panel C shows all age MPF for randomly

selected locations from the Southern and Northern regions. Panel D shows the age standardized parasite prevalence ($PfPR_{2-10}$) among the trauma cases.

3.5.4 Factors predicting malaria positive slides among admissions

Over the full monitoring period from 1990-2014 there was significant variation in MPF over space and time, and the spatial variation was most marked in the post-decline period (Figure 3.6C). The variation accounted for by an interaction between space and time was also more marked during this period, suggesting substantial temporal instability of the sub-regional spatial pattern during this period (Figure 3.8D). The interaction between space and time was strongly significant ($p<0.0001$) but accounted for only ~1% of the variability in MPF. However, the regional differences (i.e. with lower MPFs North versus South of the creek) remained temporally consistent despite the sub-regional instability.

The prevalence of ITN use among residents in the Northern region was estimated at 32%, 16% and 26% in the years 2000, 2003 and 2005, respectively (malaria indicator surveys, Table 2) and then 55.9% (95%CI 55.7 to 56.1) in 2009 rising to a high of 82.6% (95%CI 82.5 to 82.8) in 2013 during the mass ITN distribution campaign (Figure 3.9).

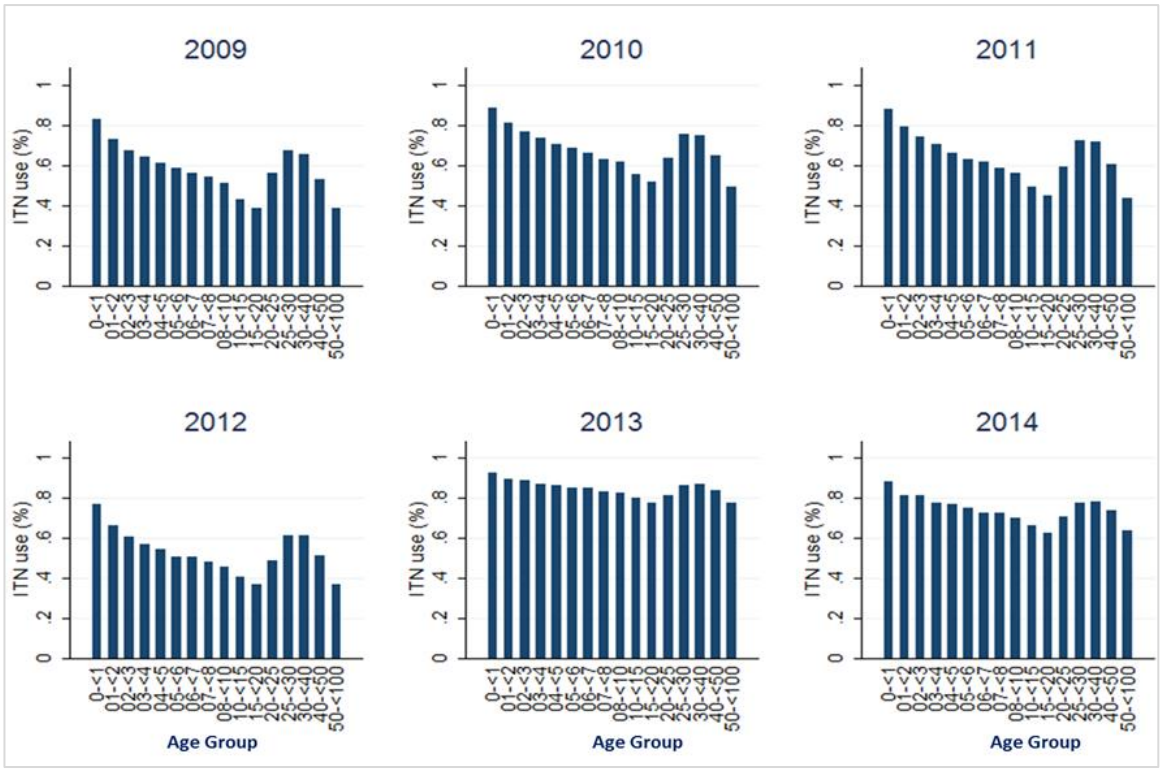

Figure 3.9*: Trends in ITN use by age group (years) over time for the period (2009 -2014).*

Table 3.2: Data from Malaria indicator surveys conducted in the Northern region of Kilifi Demographic Surveillance Area

Clusterid	Year	population size	ITN use (N)
157	2000	19	10
159	2000	11	11
160	2000	11	2
161	2000	11	0
162	2000	16	5
163	2000	16	4
164	2000	10	0
165	2000	13	1
166	2000	7	2
167	2000	13	2
168	2000	7	4
170	2000	8	0
172	2000	14	4
173	2000	11	9
174	2000	16	1
177	2000	17	3
179	2000	10	5
180	2000	12	7
1230	2000	12	4
2	2003	87	39
48	2003	118	0
86	2003	113	3
99	2003	54	27
279	2003	99	5
280	2003	129	15
286	2003	66	22
291	2003	43	7
317	2003	114	21
336	2003	112	22
349	2003	73	31
359	2003	99	0
362	2003	156	11
163	2005	33	22
167	2005	28	7
171	2005	53	7
176	2005	51	14
180	2005	68	4
259	2005	95	16
265	2005	81	6
271	2005	59	29
1418	2005	37	17
1478	2005	29	11
1482	2005	21	12

Detailed spatial data was available between 2009 and 2014 including Enhanced Vegetation Index data (EVI, a proxy for soil moisture) and ITN use for each of the 250,000 residents in the study area (Figure 3.10 and Figure 3.11).

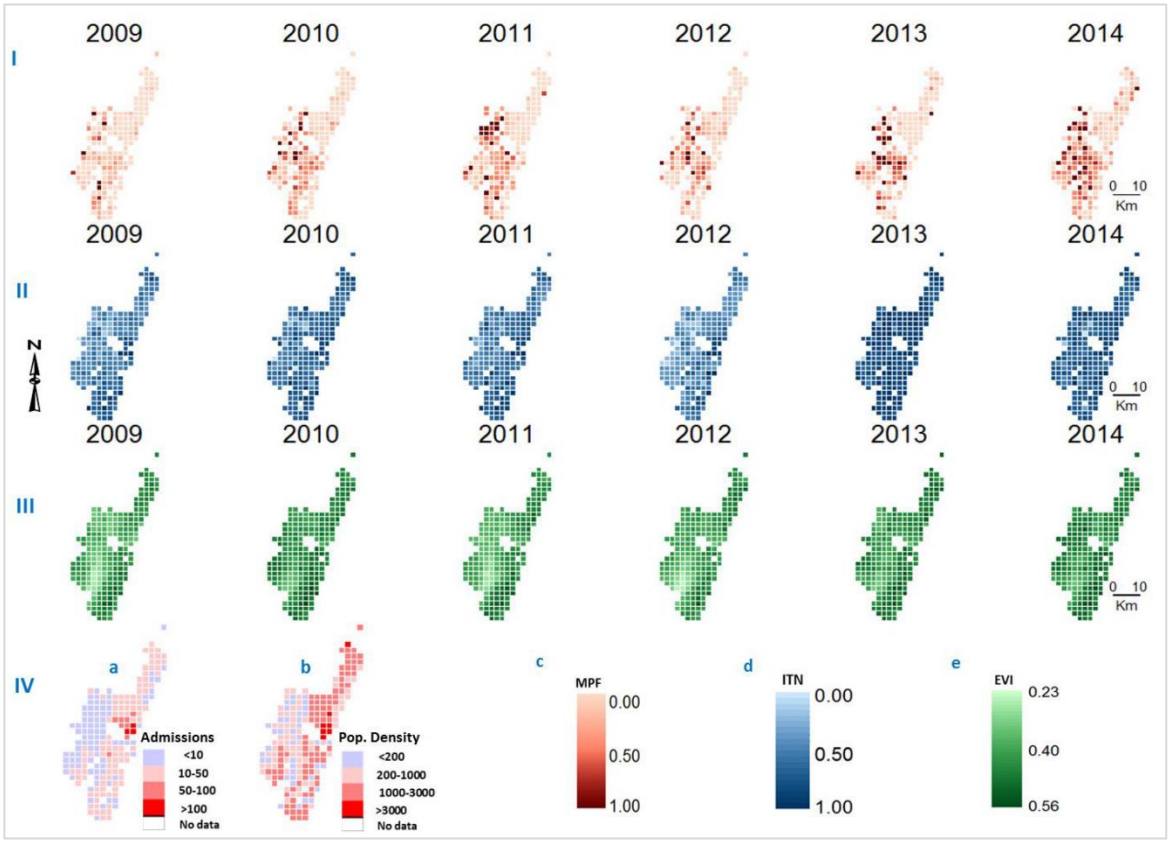

Figure 3.10*: Finer-scale geographical distribution of malaria positive fraction (row I), insecticide-treated net use (row II) and enhanced vegetation index (row III) over time and the average admissions per year (row IVa) and population density (row IVb).*

Panels IVc, IVd and IVe are the legends for row I, row II and row III respectively.

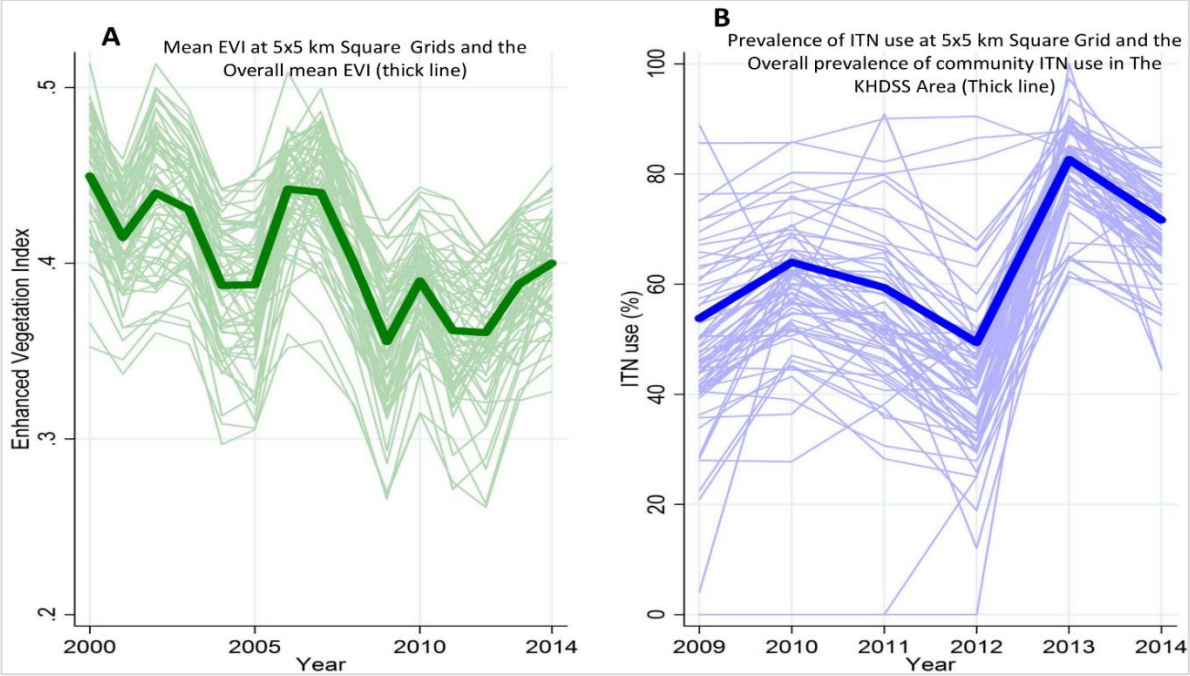

Figure 3.11*: Trends in insecticide-treated net use, enhanced vegetation index.*

Panel A shows the trends of EVI from year 2000 through to 2014. Panel B shows the trend of ITN use for a six-year period 2009 -2014.

I identified four independent predictors of malaria slide positivity; a) ITN coverage in the 2km radius around the child's residence, b) age of the child c) EVI in the 0.25x0.25 km square around the child (i.e. the finest resolution at which EVI was available) and d) time (Figure 3.12B, Table 3.3). These predictors explained ~ 23% of the variability on the outcome variable leaving about 77% unexplained variability in the response variable.

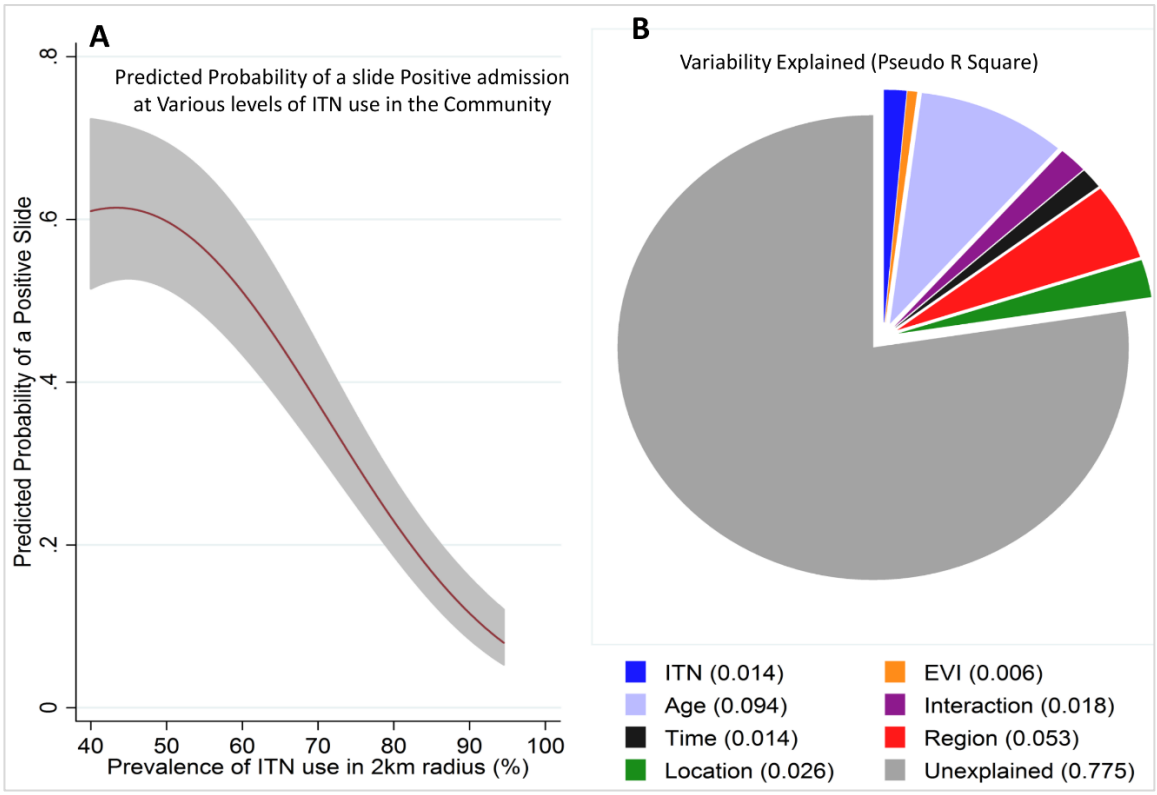

Figure 3.12*: Regression model prediction and variability explained by the predictors of the model.*

Panel A shows the predicted probability of a positive slide result (y axis) against the prevalence of ITN use in a 2km radius around each admitted child's residence. Panel B shows the pseudo R^2 of the various variables assessed in the extended model (ITN use around a child's residence, EVI, Age, Age-time interaction, Time, Region and Location).

Personal ITN use was a predictor in univariable analysis (OR=0.73, 95%CI 0.65 to 0.83, p=<0.001), but not after adjusting for ITN use in the 2km radius around the admitted children's residences (Table 3.3).

Table 3.3: Multiple logistic regression model of malaria on personal ITN use, ITN use prevalence around the child's residence age and EVI for both complete case analysis and multiple imputation.

		Complete case analysis			Imputed data analysis		
Covariates	**Covariates from Multiple Fractional Polynomial model**	**Odds Ratio**	**P-value**	**(95% CI)**	**Odds Ratio**	**P-value**	**(95% CI)**
Personal ITN use	Personal ITN use	0.925	0.307	(0.797 1.075)	0.937	0.37	(0.814 1.087)
Community level ITN use (2km radius)	ITN1=ITN_2km^2-0.3908547648	0.159	<0.001	(0.104 0.242)	0.154	<0.001	(0.102 0.233)
	ITN2=ITN_2km^2*ln(ITN_2km)+0.1835882418	2.3E-4	<0.001	(1.1E-5 5.0E-3)	4.9E-4	<0.001	(3.0E-5 8.4E-3)
Age in Years	Age1=(Age/10)^0.5-0.5888197049	3.0E+07	<0.001	(4.6E+6 1.9E+8)	1.3E+07	<0.001	(2.6E+6 6.6E+7)
	Age2=(Age/10)-0.3467086449	6.1E-06	<0.001	(1.5E-6 2.5E-5)	2.8E-05	<0.001	(8.9E-6 8.6E-5)
EVI (0.25x0.25 km)	EVI	23.43	<0.001	(4.40 124.17)	12.26	0.001	(2.73 55.00)
Time=(year-2008)	T1=ln(time)-1.142973262	2.11	<0.001	(1.77 2.43)	1.30	<0.001	(1.24 1.36)
Age-Time interaction	Age1*T1	4.2E-3	0.002	(1.3E-4 0.14)	0.32	0.01	(0.14 0.76)
	Age2*T1	120.56	<0.001	(8.3 1.8E+3)	2.44	0.003	(1.35 4.40)

There were no marked differences in characteristics of missing ITN data compared to non-missing data by age, EVI, gender and location of residence (Table 3.4). Results from multiple imputation were not significantly different from complete case analysis (Table 3.3).

Table 3.4: Characteristic of individuals with missing ITN data during community ITN use surveys.

Characteristics		**Missing data**	**Complete data**
Overall	No. (%)	190,411 (12.81)	1,296,346 (87.19)
Age (years) median (iqr)		19 (8 - 31)	15 (7 - 32)
EVI median (iqr)		0.366 (0.326-0.393)	0.370 (0.336-0.397)
Male	No. (%)	75,823 (48.16)	501,641 (46.70)
Missing by locations			
Chonyi	No. (%)	28,178 (14.80)	215,829 (16.65)
Gede	No. (%)	14,649 (7.69)	108,598 (8.38)
Jaribuni	No. (%)	7,904 (4.15)	70,616 (5.45)
Junju	No. (%)	23,470 (12.33)	150,654 (11.62)
Mtwapa	No. (%)	9,870 (5.18)	63,074 (4.87)
Ngerenya	No. (%)	9,834 (5.16)	79,737 (6.15)
Roka	No. (%)	11,714 (6.15)	87,215 (6.73)
Sokoke	No. (%)	5,251 (2.76)	44,089 (3.40)
Takaungu	No. (%)	16,751 (8.80)	137,710 (10.62)
Tezo	No. (%)	62,790 (32.98)	338,822 (26.14)

There was strongly significant ($p<0.0001$) non-linear relationship between community ITN use in the 2km radius area around a child's residence and a slide positive result such that MPF reduced substantially with increasing ITN use (Figure 3.12A). The probability of presenting for admission with clinical malaria increased with higher values of EVI around the child's residence and with later years of admission. However, the probability of clinical malaria increased to a peak at ~5-years of age at admission and decreased again (Figure 3.13). Similar results were obtained using a model that included clustering at the 250 square meters (Table 3.5) except that the confidence intervals were slightly wider (Figure 3.13).

Table 3.5: Multiple logistic regression model of malaria on personal ITN use, ITN use prevalence around the child's residence and EVI (allowing clustering at 250 square meters)

Covariates	**Covariates from multiple fractional polynomial model**	**Odds ratio**	**P-value**	**(95% CI)**
Personal ITN use	Personal ITN use	0.925	0.355	(0.78 1.09)
Community level ITN use (2km radius)	ITN1=ITN_2km^2-0.3908547648	0.159	<0.001	(0.097 0.259)
	ITN2=ITN_2km^2*ln(ITN_2km) +0.1835882418	2.3E-4	<0.001	(6.6E-6 0.008)
Age in Years	Age1=(Age/10)^0.5-0.5888197049	3.0E+07	<0.001	(4.0E+6 2.3E+8)
	Age2=(Age/10)-0.3467086449	6.1E-06	<0.001	(1.3E-6 2.9E-5)
EVI (0.25x0.25 km)	EVI	23.4	<0.003	(2.86 190.7)
Time=(year-2008)	T1=ln(time)-1.142973262	2.1	<0.001	(1.76 2.44)
Age-Time interaction	Age1*T1	4.2E-3	0.004	(1.0E-4 0.17)
	Age2*T1	120.56	0.001	(7.1 2.0E+3)
	Pseudo R^2 =0.144			

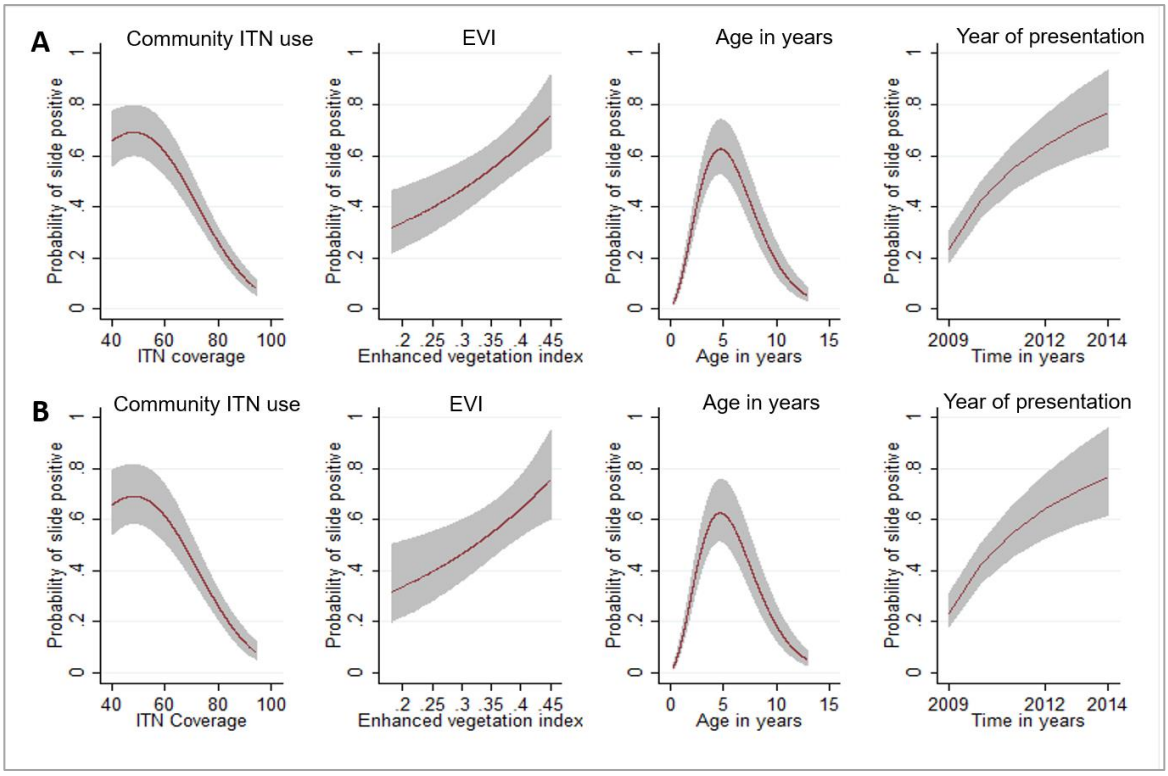

Figure 3.13: MFP regression model prediction of all predictors.

Row A (without accounting for clustering) and B (accounting for clustering) shows the predicted probability of a positive slide result (y axis) against the predictors included in the model. Variables assessed in the extended model are ITN use 2km radius around a child's residence, the enhanced vegetation index (EVI), age in years and time (year of presentation to hospital for admission).

3.5.5 Sensitivity Analysis

It has previously been shown that applying a parasite threshold to the definition of malaria enhances the specificity of the endpoint, as lower parasite densities are more likely to reflect coincident chronic asymptomatic parasitaemia and a non-malarial clinical illness such as pneumonia. I therefore repeated the analysis using a cut-off >2,500 parasites per µl. The same patterns were seen: i.e. a post-decline increase in MPF among older children (Figure 3.14), and

inverse relationship between MPF and age of malaria (r=-0.63, p<0.001), and of protection by community-level ITN use (Figure 3.15). These patterns remained statistically significant.

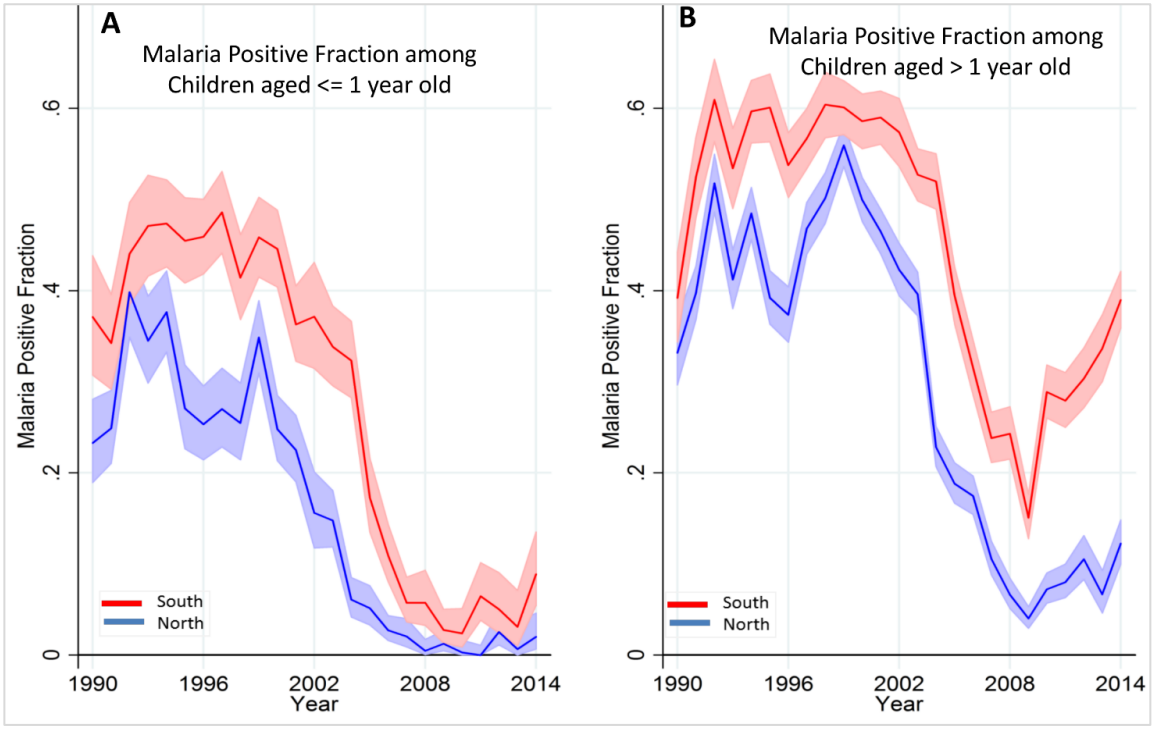

Figure 3.14: Trends of malaria positive fraction, over time and region using a case definition for malaria of >2,500 parasites/µl.

Panels A and B show the temporal trends of MPF in admitted children aged<=1-year-old and children aged>1-year-old respectively, red line represents the Southern region while the blue line represents the Northern region of the hospital.

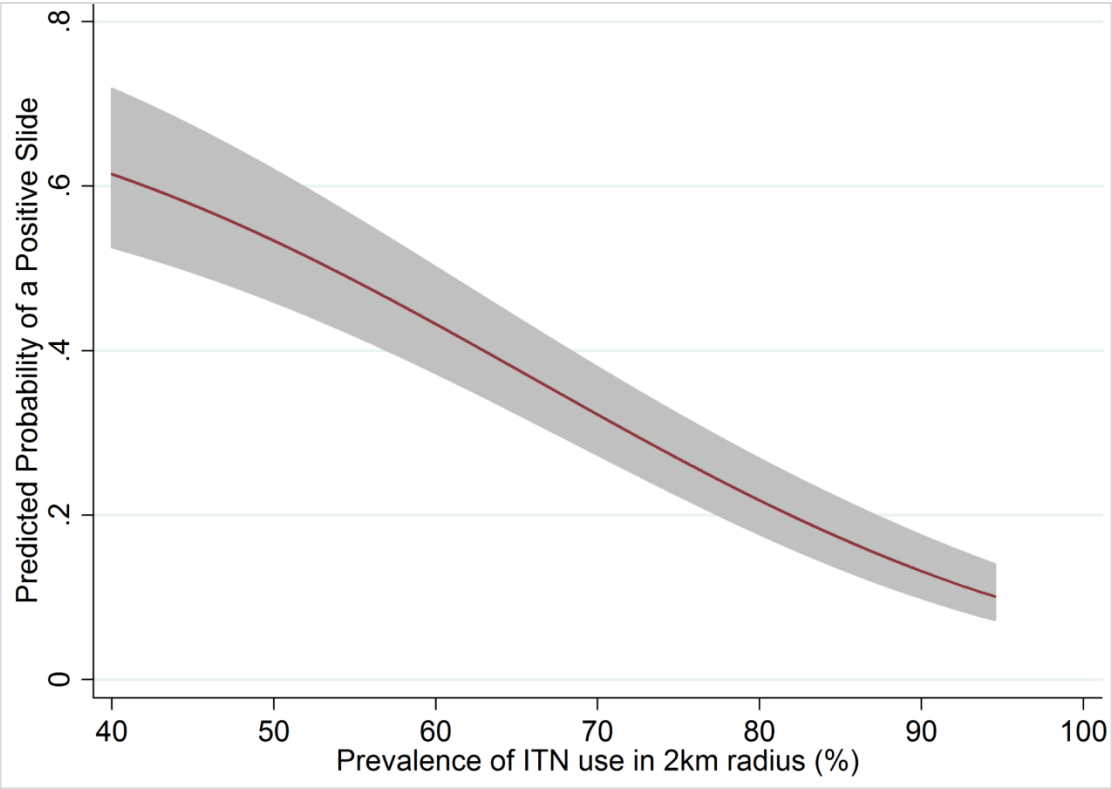

Figure 3.15: Regression model prediction using a case definition for malaria of >2,500 parasites/µl.

The figure shows the predicted probability of a positive slide result (y axis) against the prevalence of ITN use in a 2km radius around each admitted child's residence.

3.6 Discussion

The proportion of acutely unwell children admitted to hospital with malaria positive slides (i.e. the MPF) declined from 2002 until 2009 (as reported previously *(O'Meara et al. 2008)*). However, after 2009 there was an increase in MPF that continued through to 2014. This increase was mostly seen in older children: there were relatively modest increases among children less than 1 year of age ($MPF_{<1yr}$), but much greater increases among children above 1 year of age ($MPF_{>1yr}$). This combination of findings suggests that the increase in malaria is due to increasing susceptibility to disease among older children, exacerbated by a slight increase in underlying transmission intensity since 2009. Additional evidence for increasing transmission after 2009 can be seen in the increasing prevalence of asymptomatic parasitaemia (PfPR) among children admitted for trauma, and work showing increasing parasite prevalence throughout the Kenyan Coast *(Snow et al. 2015)*.

It has been shown previously that at high transmission intensities children acquire immunity rapidly, and so are not susceptible to disease when they are older *(Okiro et al. 2009)*. On the other hand, at low transmission intensity there is less disease among younger children, and consequently older children acquire less immunity and remain susceptible *(Okiro et al. 2009)*. Therefore, the lowest malaria rates will likely occur just after a recent reduction in transmission: at this point current exposure is low, but older children are immune because of their previous exposure when transmission was high. As time goes by after a reduction in transmission, a cohort of older children will emerge who are susceptible to the disease, and the rates of malaria will increase again.

Taking together these finding of a) trends of increasing age of children admitted with positive malaria slides in parallel with falling $PfPR_{2-10}$ in the community and falling $MPF_{<1}$ among

hospital admissions b) a geographical association where areas with high MPF have low average age of children admitted with malaria c) previous analyses *(Reyburn et al. 2005)* and geographical analyses *(Snow et al. 1997)* showing the importance of the relationship between prior exposure and immunity to malaria, the most likely conclusion is that reduced exposure led to increasing susceptibility among older children and hence the subsequent increase in MPF.

Other potential explanations for increasing malaria admissions among older children could include increased prevalence of preferential ITN use among younger children rather than loss of immunity. However, multivariable models that accounted for personal ITN use showed that age, and the interaction between age and year, were independent predictors of malaria, indicating that the increasing MPF among older children was independent of variation in ITN use by age (Table 3.3 and Table 3.5). Furthermore, the difference in ITN use by age was modest (87% of children less than 1 year of age used ITNs compared with 78% of children between 1 and 5 years of age). Other interventions to prevent malaria targeted at younger children that increased over time could produce a similar pattern, but there are no such interventions reported in the area. An increasing prevalence of outdoor biting might also preferentially affect older children. However, this would not explain the clear inverse correlations seen between MPF and average age over time and by geographical region, which suggests that immunity is an important component of the variation in MPF. In the absence of host immunity older children may be at higher risk than younger children since mosquito biting may be proportional to surface area *(Smith et al. 2004)*. However, MPFs do not necessarily reflect incidence. For instance, the risk of non-malarial fever falls as children get older which would lead to an apparent increase in MPF.

The scale-up in ITN use occurred in 2006, and therefore is not related to the reductions in transmission seen from 2000 onwards: changing anti-malarial drug use and rainfall may be relevant *(Snow et al. 2015)*. Regardless of the cause of the prior decline, I found clear evidence of ITN efficacy during the period in which they were used. Personal ITN use was associated with reduced MPF among children admitted to hospital. However, when multivariable models were examined it was the prevalence of ITN use in the 2km radius area around a child that independently reduced the probability of a malaria positive slide, regardless of personal use. Hence it is likely that the resurgence in malaria seen since 2009 would have been even more marked in the absence of ITNs.

The WHO and Kenyan national policy recommend universal ITN coverage to control malaria. However, operational constraints often do not allow this and so instead many distribution programmes target younger children and pregnant women as high risk groups. Our data suggest that universal coverage should be a high priority since a) older children are increasingly in need of protection as transmission falls and b) the mass-protective effect would achieve substantial gains in malaria control above those achieved by personal ITN use.

ITN use was lowest among adolescents and young adults (Figure 3.9). The low ITN use among 10-15-year-old adolescent girls is a cause of concern as they transition to young mothers (primigravidae). Primigravidae are at a particular risk of severe malaria and are at a higher risk of stillbirth, miscarriage, preterm delivery low birthweight and increased infant mortality when exposed to malaria *(Brabin 1983, Guyatt and Snow 2001, Conroy et al. 2012)*. First pregnancies (primigravidae) are at the highest risk of infection and adverse pregnancy outcomes because of lack immunity to 'pregnancy-specific' variants of *Plasmodium falciparum* that selectively accumulate in the placental intervillous space leading to placental and occult placental malaria

that may not always be associated with detectable blood-stage infection *(Conroy et al. 2012)*. Community engagement and sensitization targeting this age groups should be conducted to enhance coverage.

Fortunately, there was no strong evidence of an increasing case fatality rate as transmission fell (Figure 3.5D). The risk of mortality from severe malaria increases in older age-groups across the range from children to adults *(Dondorp et al. 2008)* but this increase may be less evident within the age range of children below 10 years of age *(Marsh et al. 1995, von Seidlein et al. 2012b)*. Furthermore, many other factors influence mortality over time, including variation in hospital acceptance over time, access to care (due to improved road network), better care *(Dondorp et al. 2010, Maitland et al. 2011)* and socioeconomic conditions. My data do not suggest that increasing mortality is an immediate concern of the post-decline period despite the increasing rates of malaria in older children.

Study limitations include the use of MPF among acute admissions which may be biased by varying access to care. In support of the use of MPF, there was a close correlation between location-matched MPFs with the incidence rates from active case detection data from two cohort studies conducted within the same study area. Furthermore, geographic heterogeneity was larger than any consistent bias in MPF with distance to the hospital (Figure 3.4), agreeing with previous work *(Bejon et al. 2014)*.

The analyses were based on the proportion of children with positive malaria slides. This case definition includes children with chronic asymptomatic parasitaemia and coincident fever. To exclude a bias resulting from including these children I repeated the analysis after applying a threshold of >2,500 parasites per µl, which excludes the majority of children with asymptomatic parasitaemia *(Bejon et al. 2007)* (Figure 3.14 and 3.15).

The ITN use surveys were conducted annually and based on reported rather than observed use. The potential misclassification of this approach could lead to an underestimate of the protective effect of ITNs. During the study period, there were no other widespread interventions such as anti-malarial prophylaxis or presumptive treatment that were targeted at younger children. ITN use has not been randomly assigned and therefore confounding could be present. If present, this would most likely dilute the effect size (i.e. ITN use would be better taken up in areas of higher malaria risk). Furthermore, observational studies of ITN use have consistently shown similar findings to randomized controlled Trials (RCTs) suggesting that within specific communities confounding is not pronounced *(Lim et al. 2011)*.

Our data were taken from a single geographical setting. However, there is evidence that immunity to malaria is dependent on exposure in a wide range of geographical settings *(Okiro et al. 2009)* and therefore our findings may be relevant more widely across Africa in settings where transmission is falling *(O'Meara et al. 2010)*.

In conclusion, I show that despite substantial reductions in malaria transmission in Kilifi, residual malaria transmission has continued and older children are increasingly vulnerable to disease. As countries and regions make progress in malaria control *(Noor et al. 2014)*, maintaining control measures will be essential: in fact, further progress will be required to offset the increasing rates of malaria in older children. Achieving ITN coverage close to 100% shows promise as a strategy, but will require novel strategies, particularly among groups that seem less keen to use ITNs such as adolescent males and young adults *(Noor et al. 2009b)*.

Chapter Four

4 Effect of transmission intensity on hotspots and micro-epidemiology of malaria in sub-Saharan Africa

4.1 Introduction

In chapter 3 above I demonstrated that heterogeneity of malaria transmission increases with decreasing transmission intensity. This concept has been previously derived using mathematical models but little empirical evidence is available.

Here I sought to provide further empirical evidence on the trends in micro-heterogeneity of malaria transmission using a large assembled set of data from studies conducted in sub-Saharan Africa on a) acute symptomatic malaria (detected through active and/or passive case detection or cross-sectional surveys) and b) prevalence of parasitaemia detected through cross-sectional surveys *(Mogeni et al. 2017a).*

4.2 Objectives

I used data drawn from 19 different study sites across 7 sub-Saharan African countries, representing a range of transmission intensities from intense transmission in Burkina Faso *(Tiono et al. 2013)* to low transmission in The Gambia and the Northern part of Kilifi, Kenya *(Mogeni et al. 2016)* to :-

1. Describe trends in parameters describing local clustering (or hotspots) and in global measures of spatial autocorrelation of malaria cases at varying transmission intensities.
2. Compare temporal stability of malaria hotspots
3. Examine the association between micro-variations in mean age of symptomatic malaria (as a proxy for exposure/acquired immunity) and the MPF across the sites.

4.3 Methods

4.3.1 Multisite data collation from centers in sub-Saharan Africa

A group of researchers interested in malaria hotspots work were approached to participate in forming a group (The Hotspot Group) and make available homestead level geocoded datasets with the aim of assessing the properties of malaria hotspots in sub-Saharan Africa. An email invitation was sent to the Hotspots Group members inviting them to make available data on asymptomatic parasitaemia or febrile malaria surveillance or both and including age of study participants and homestead level GPS co-ordinates for any length of monitoring. All the investigators contributing data were assured of being listed in any resulting manuscript(s) and their contribution acknowledged.

To identify studies assessing microepidemiology of malaria transmission, a search was conducted in PubMed on the geographical heterogeneity of malaria transmission, variability of malaria risk by age and stability of hotspots using MeSH terms “Childhood” “Malaria” (“Heterogeneity” OR “Heterogeneous” OR “Variability” OR “Variation” OR “Hotspots”) and “Age”. Several previous studies assessed the properties of hotspots of malaria transmission in single sites. Few studies examined stability of hotspots and only one study investigated the efficacy of targeting control interventions on hotspots of malaria transmission. There were no previous studies examining the properties of hotspots across multiple sites.

Studies that used microscopy for detection of malaria parasites, performed clinical assessments for presence or absence of fever, reported homestead level geospatial coordinates and age of study participants were included in the analyses. For cluster-randomised or individual-randomised controlled trials, data from intervention and control arms were analysed separately. Datasets were then further subdivided by year before analysis for spatial clustering. Only studies

where Ethical approval and consent for human participation had been granted by authorities of the participating countries were reported. In the Kilifi sites, approval for human participation in cross-sectional surveys, cohorts and hospital surveillance was given by the Kenya Medical Research Institute Ethics Research Committee. Prior to any study procedure, written informed consent was obtained from all individuals participating in the surveys or, where appropriate, their parents or guardians. The studies were conducted per the principles of the declaration of Helsinki.

4.3.2 Data

Data were assembled from studies conducted in sub-Saharan Africa (Figure 4.1, Table 4.1) with homestead-level geospatial records linked to malaria surveillance at sites with varying transmission intensities. Data were shared with no personal identifiers except geospatial coordinates.

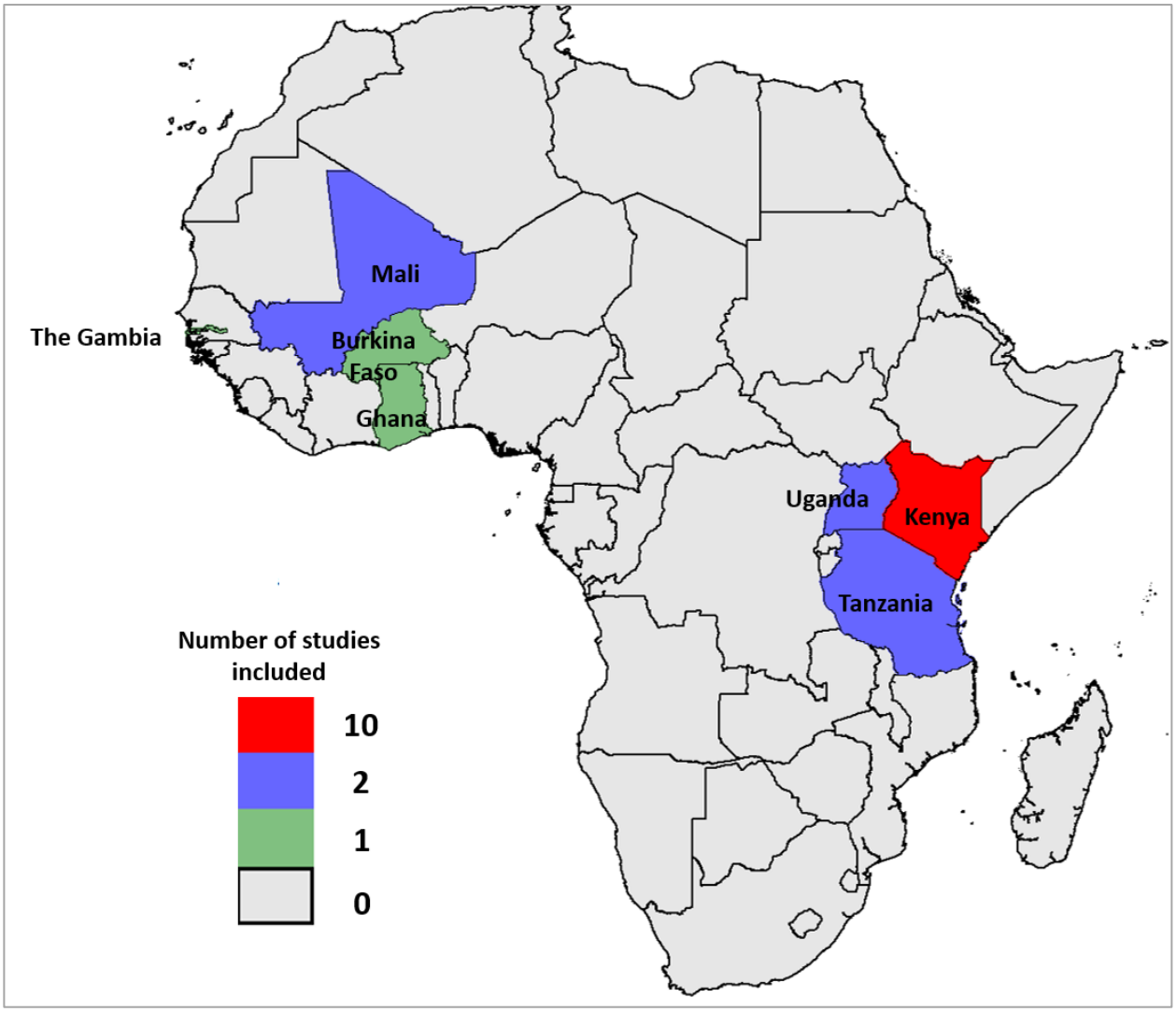

Figure 4.1: ***Map of sub-Saharan Africa showing countries and their respective number of studies included in the analysis***

4.3.3 Malaria case definition

Symptomatic malaria and asymptomatic parasitaemia were classified per the definitions shown in Table 4.1. The key metrics were *P. falciparum* parasite prevalence (i.e. the proportion of asymptomatic parasite carriage from community cross-sectional surveys), malaria positive fraction (MPF; defined as the fraction of symptomatic malaria) and mean age of children presenting with symptomatic malaria.

Table 4.1: Study characteristics

Location	Study description	Sample size (N)	Malaria case definition (symptomatic malaria)	MPF	Asymptomatic parasitaemia assessed?	Parasite Prevalence (%)
Kilifi Kenya *(Bejon et al. 2014)*	Ngerenya Dispensary Surveillance; monitoring was conducted from 1st April 2014 to 31st December 2015	1998	Any presentation with parasitaemia	0.048	No	Not applicable
Kilifi Kenya *(Bejon et al. 2010)*	Junju cohort, monitored between 1st January 2005 and 31st December 2015	4534	Temperature >37.5 ºC and parasitaemia >2500/µL	0.376	No	Not applicable
Kilifi Kenya *(Bejon et al. 2010)*	Ngerenya cohort, monitored between 1st January 2003 and 31st December 2015	3659	Temperature >37.5 ºC and parasitaemia >2500/µL	0.043	No	Not applicable
Kilifi Kenya *(Kangoye et al. 2016)*	Ganze RTSS cross-sectional surveys of asymptomatic parasitaemia and a study cohort monitored for clinical episodes in 2012 and 2013	2532 1518*	Temperature >37.5 ºC and parasitaemia >2500/µL	0.053	Yes	1.25
Kilifi Kenya *(Bejon et al. 2014)*	Pingilikani Dispensary Surveillance; monitoring was conducted from 1st January 2009 to 31st December 2014. Each year's data were analysed separately to capture temporal trend in transmission intensity	22595	Temperature >37.5 ºC and parasitaemia >2500/µL	0.243	No	Not applicable
Kilifi Kenya *(Mogeni et al. 2016)*	Kilifi County Hospital Surveillance; monitoring conducted from 1st January 2009 to 31st December 2014. Each year's data were analysed separately to capture temporal trends in transmission intensity	8707	Any slide positive test result among acute admissions	0.171	No	Not applicable

Kilifi, Kenya *(Bejon et al. 2007)*	Junju cross-sectional bleeds between 2011 and 2015, each year's data were analysed separately to capture temporal trends in transmission intensity	1925	Not applicable	_	Yes	16.05
Nandi, Western Kenya *(Brooker et al. 2004)*	10-week active case surveillance study undertaken in three schools in Nandi District, Western Kenya during a malaria outbreak May to July 2002	520	Temperature >37.5 ºC and parasitaemia >2500/µL	0.242	No	Not applicable
Western Kenya *(Ernst et al. 2006)*	Hospital surveillance study conducted between 2001 - 2004.	599	Temperature >37.5 ºC and parasitaemia >2500/µL	0.084	No	Not applicable
Asembo, Western Kenya *(Lindblade et al. 2004)*	In late 1996, villages in Asembo were randomized into intervention and control villages. Cross-sectional surveys were conducted between 1996 and 2001. Data from symptomatic and asymptomatic individuals were analysed separately and by year of enrollment.	3614 3047*	Measured axillary temperature >37.5 ºC and parasitaemia >2500/µL	0.659	Yes	61.9
Rural Afigya-Sekyere, Ghana *(Kreuels et al. 2008)*	Cohort of infants monitored by monthly active case detection and passive case detection. Enrolled at 3 months (±4 weeks) of age between January 2003 and September 2005. Treatment and placebo arms were analyzed separately.	2721	Temperature >37.5 ºC and parasitaemia >500/µL	0.413	No	Not applicable

Mulanda, Eastern Uganda *(Pullan et al. 2010)*	Cross -sectional study conducted in four contiguous villages in Mulanda, sub-county in Tororo, Eastern Uganda between July and December 2008. age	985	Not applicable	_	Yes	53.7
Uganda *(Kamya et al. 2015)*	Cohort study of three Uganda sub-counties (Nagongera, Walukuba and Kihihi) between 2011 and 2014	3239	Temperature >37.5 ^{o}C and parasitaemia >2500/µL	0.331	No	Not applicable
The Gambia *(Hennig et al. 2015)*	Cohort study of four Gambian villages (Keneba, Manduar, Jali and Kantong Kunda) between 2009 and 2012	3117	Temperature >37.5 ^{o}C and parasitaemia >2500/µL	0.024	No	Not applicable
Mali *(Sissoko et al. 2015)*	Cross-sectional surveys were conducted during the wet and dry seasons and passive case detection in two villages in Mali were conducted between May (Kolle) or July (Sotuba) and December 2009	1867 1128*	Temperature >37.5 ^{o}C and parasitaemia >2500/µL	0.424	Yes	15.61
Mali *(Crompton et al. 2008)*	Longitudinal study conducted between May and December 2006. Analysis was restricted to children aged 2-15 years	695 695*	Temperature >37.5 ^{o}C and parasitaemia >2500/µL	0.51	Yes	21.75
Tanzania *(Mosha et al. 2013)*	Cross sectional survey conducted between August and November 2010 in northern Tanzania. Analysis was restricted to children <15 years	328	Not applicable	-	Yes	52.23

Northern Tanzania *(Gosling et al. 2009)*	The study was conducted between July 2004 and July 2007. Infants aged 2-4 months randomized to treatment regimens. Treatment and placebo arms were analyzed separately.	2300	Temperature >37.5ºC and parasitaemia >2500/µL	0.161	No	Not applicable
Saponé district, Burkina Faso *(Tiono et al. 2013)*	Cluster-randomized study with treatment and control arms. Four cross-sectional surveys were conducted between January 2011 and January 2012: a) before randomization, b) 1 month, c) 2 months and d) 12 months. Monitoring for symptomatic malaria was conducted passively at local health care facilities during the same study Period. Treatment and placebo arms were analyzed separately.	4045 11932*	Temperature >37.5 ºC and parasitaemia >2500/µL	0.707	Yes	31.32

** Shows sample size for asymptomatic parasitaemia studies when both symptomatic and asymptomatic datasets were available for analysis*

4.3.4 Statistical methods

Data from each site were used to quantify spatial clustering of malaria (described in detail above). The various metrics from each site were pooled together to examine systematic variation in metrics of spatial clustering over transmission intensity using site as the unit of analysis. Observations with missing geo-coordinates, age and malaria slide results in any of the requested datasets were excluded prior to the analysis. No data imputation was done at any analysis stage.

4.3.5 Local cluster detection

The spatial scan statistic (details are discussed in chapter 2) was used to detect local spatial clusters of asymptomatic carriers and/or symptomatic malaria cases using a Bernoulli model. Cases were individuals with malaria and controls were individuals without malaria. I assessed variation in the number of hotspots per study site, the risk ratio (RR) of the most likely (primary) hotspot (i.e. the ratio of the risk of malaria within the hotspot divided by the risk outside the hotspot) and the p-value of the primary hotspot over transmission intensity.

4.3.6 Global spatial pattern analysis

Ripley's K-function was used to analyse binary data and Moran's I for the analysis of the age of children with clinical malaria as a measure of acquired clinical immunity. The K-function and the Moran's I methodology have been described in detail in chapter 2. Key parameters of interest from this analysis were the estimate of the D-function, the spatial autocorrelation coefficients and measures of significance. Sensitivity analysis was conducted at various predefined distances.

Symptomatic malaria cases and asymptomatic parasitaemia were examined separately. For each dataset, parameters from the local cluster detection and from the D-function analyses were

assessed against the overall transmission intensity measured by the MPF for datasets on symptomatic malaria or parasite prevalence for datasets on asymptomatic parasitaemia.

Multiple fractional polynomials to assess nonlinear fits of MPF or parasite prevalence on the hotspots parameters (i.e. number of hotspots, risk ratios, and p-values) in the regression models adjusted for potential confounders (i.e. study design, sample size and overall mean age of study participants included in each study).

4.3.7 Temporal stability of hotspots analysis

There were few datasets with repeated sampling of overlapping homesteads, and therefore stability of spatial heterogeneity could only be tested in 4 datasets from western Kenya *(Hawley et al. 2003)*, Ghana *(Kreuels et al. 2008)*, Burkina Faso *(Tiono et al. 2013)* and Uganda *(Kamya et al. 2015)*. MPFs and/or parasite prevalence were computed by grids (2x2km square) and by year (or time points for cross-sectional surveys). I assessed stability of the spatial heterogeneity by examining correlation between MPFs or parasite prevalence within grids separated in time.

4.3.8 Correlation between age of clinical malaria and transmission intensity

The average age of children with malaria was computed as the geometric mean age of children presenting with symptomatic malaria. The correlation between the average age of symptomatic malaria and MPF at predefined square grid sizes (i.e. $1km^2$, $2km^2$ and $4km^2$) were calculated using the Spearman's rank correlation coefficient. Variable grid sizes were used for sensitivity analysis and were calculated using longitude and latitude coordinates. Pooled correlations for the predefined grid size were estimated in a fixed effect meta-analysis, however if heterogeneity (I^2) between studies was large (>50%), a random-effect meta-analysis was conducted (details on meta-analyses are discussed in chapter 2). Global spatial autocorrelation for age of

symptomatic malaria at homestead level within sites was assessed using Moran's I statistic and the significance determined using Monte Carlo simulations.

SaTscan was executed from R using rsatscan package which allows SaTscan to be executed in the background from R's command line. The K-function and the Moran's I statistics were executed in R version 3.3.1, and graphs, meta-analyses, multiple fractional polynomial procedure and other analyses were conducted in Stata version 12 (StataCorp, Texas).

4.4 Results

4.4.1 Malaria morbidity in the study sites

Nineteen studies conducted between 1996 and 2015 in seven countries were examined (Figure 4.1). The characteristics of each study population are presented in Table 4.1 with references to previously published work.

4.4.2 Hotspots of malaria cases

Data from cross-sectional surveys in Asembo western Kenya, were analysed from 79 villages randomized to receive insecticide-treated bednets. In this high transmission site (overall, MPF=0.66 and parasite prevalence=61.9%), hotspots of malaria transmission were identified in both asymptomatic and symptomatic datasets, but the significance was borderline and the risk ratio slightly higher than 1. Figure 4.2 and Figure 4.3 illustrates hotspots of febrile malaria and hotspots of asymptomatic parasitaemia respectively for homesteads in the control group.

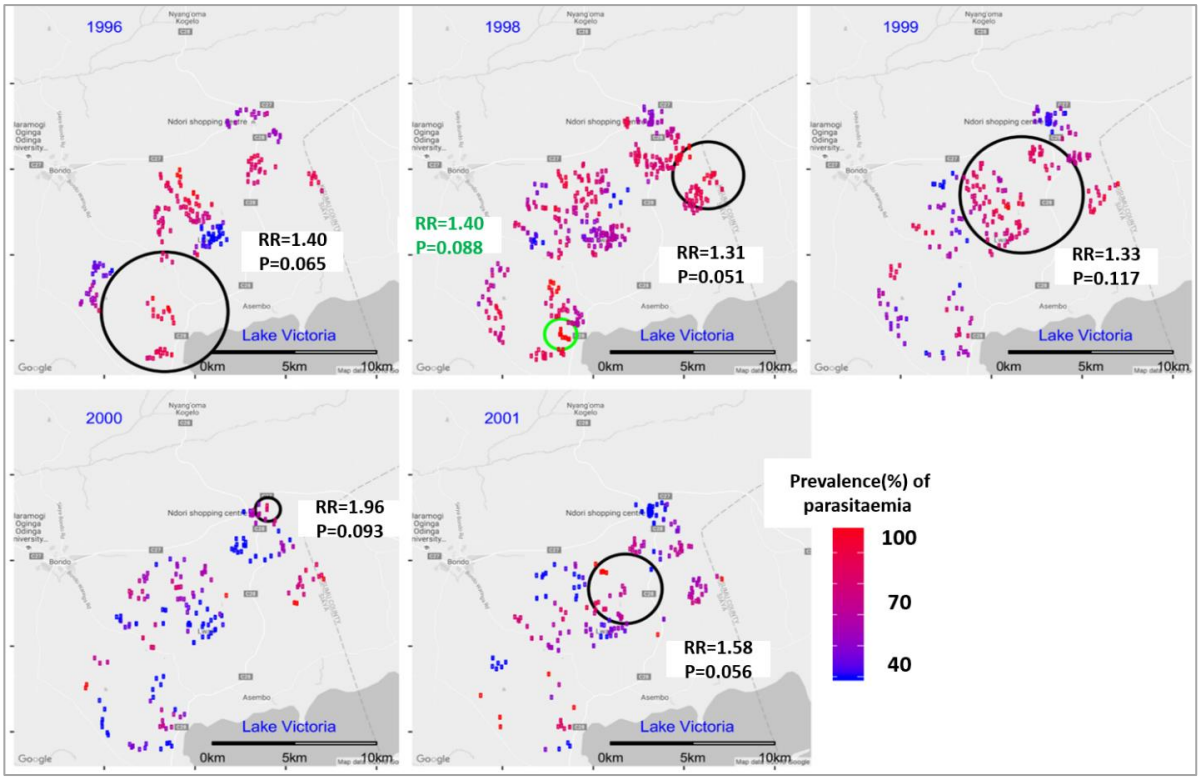

Figure 4.2*: Hotspots of asymptomatic parasitaemia cases (control arm) in Asembo western Kenya.*

*B*lack and green circle *represent statistically significant primary and secondary hotspots respectively with their relative risk (RR) and p value displayed beside the circles.*

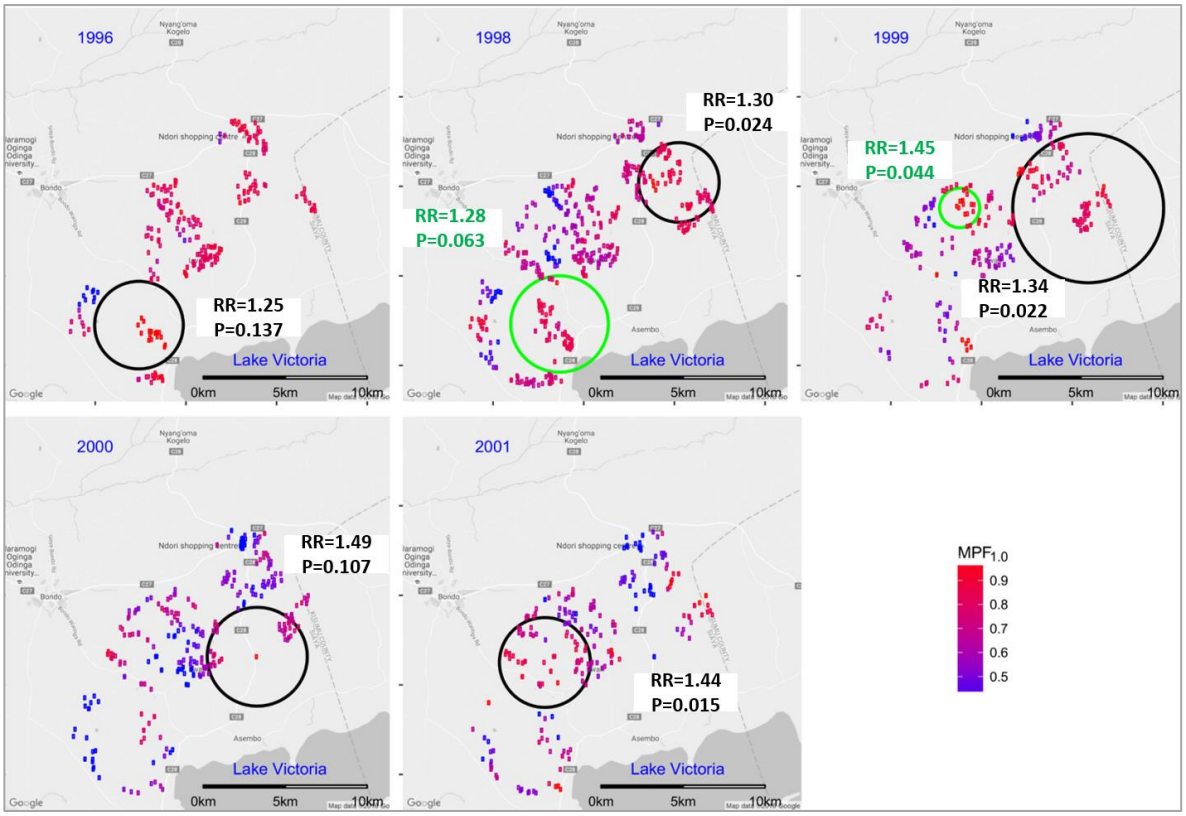

Figure 4.3*: Hotspots of clinical malaria cases (control arm) in Asembo western Kenya.*

*B*lack and green circles *represent statistically significant primary and secondary hotspots respectively with their relative risk (RR) and p value displayed beside the circles.*

Data from Sapone, Burkina Faso were analyzed from 1550 homesteads in 18 villages. Overall there were 6,848 (56.8%) episodes of febrile malaria recorded during the 12-month passive surveillance and 8,346 (25.7%) episodes of microscopically confirmed asymptomatic infections recorded during the four survey time points over the 12-month period. Hotspots of malaria transmission were identified in both asymptomatic and symptomatic datasets for both the treatment and control group. However, the significance (as illustrated by the p-values) ranged between borderline to no significance and the risk ratio were close to 1. Figure 4.4 below illustrates hotspots of febrile malaria during the months coinciding with the 4-asymptomatic malaria survey time points.

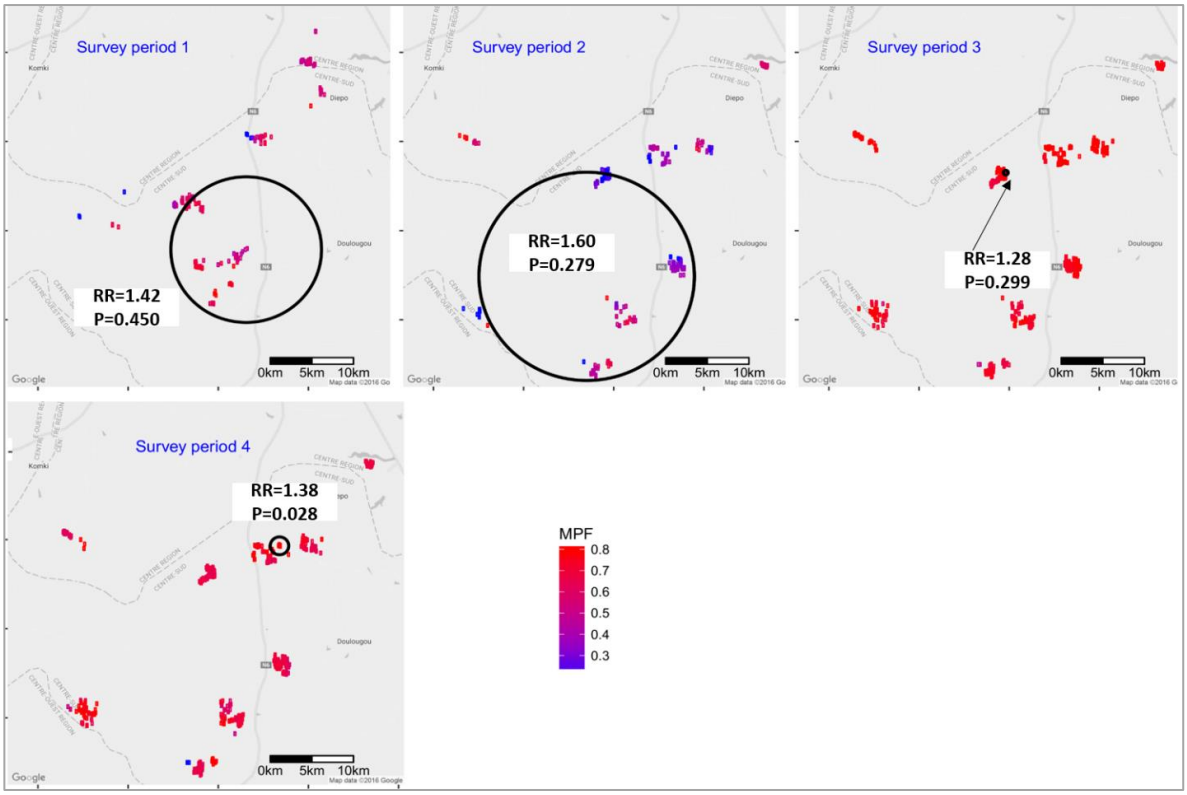

Figure 4.4*: Hotspots of clinical malaria cases (control arm) in Burkina Faso.*

Each black circle *represents a statistically significant hotspot with its relative risk (RR) and p value displayed beside the circle.*

In the low transmission settings in Kilifi and The Gambia, significant hotspots were observed in Ganze Kilifi county (overall MPF=0.05) but not in The Gambian villages (overall MPF=0.024). In contrast with the high transmission settings in Asembo and Burkina Faso (Figure 4.2 - Figure 4.4), the risk ratios were markedly different from 1 (Figure 4.5 and Figure 4.6).

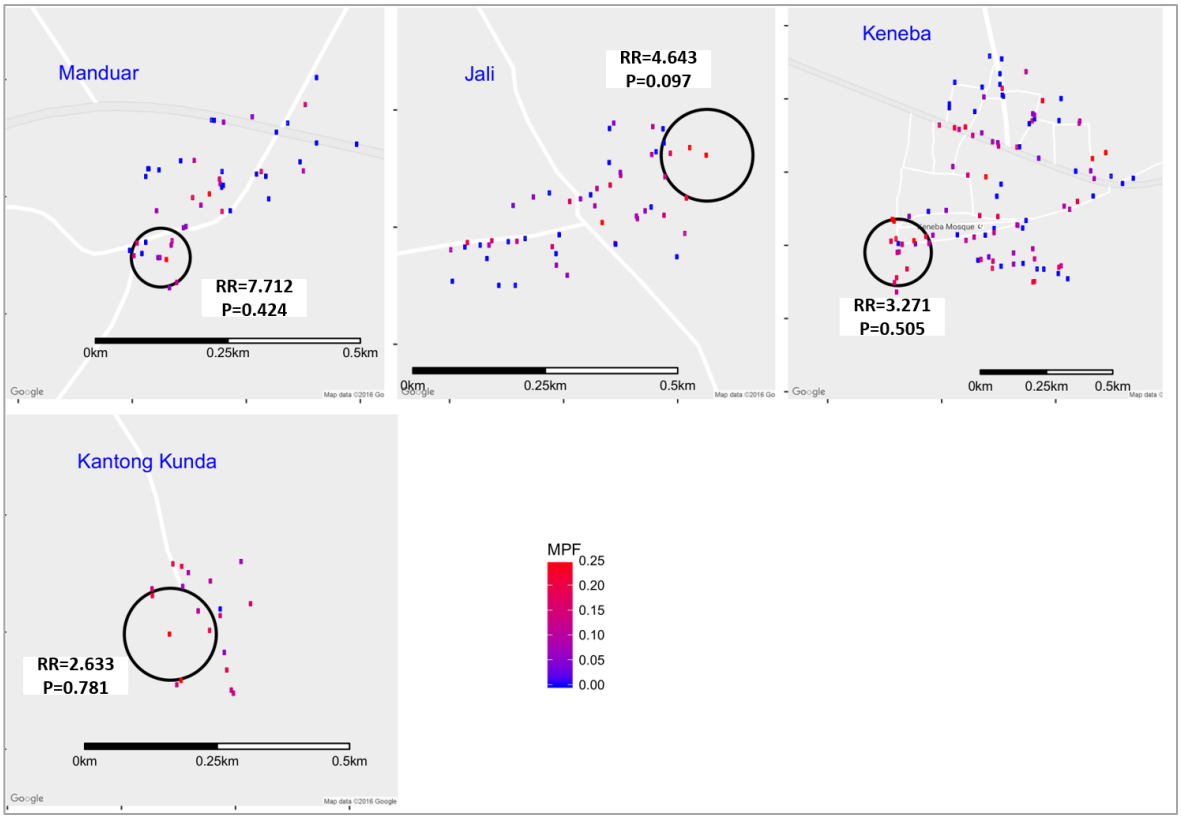

Figure 4.5*: Hotspots of clinical malaria cases by village in The Gambia.*

Each black circle *represents a statistically significant hotspot with its relative risk (RR) and p value displayed beside the circle.*

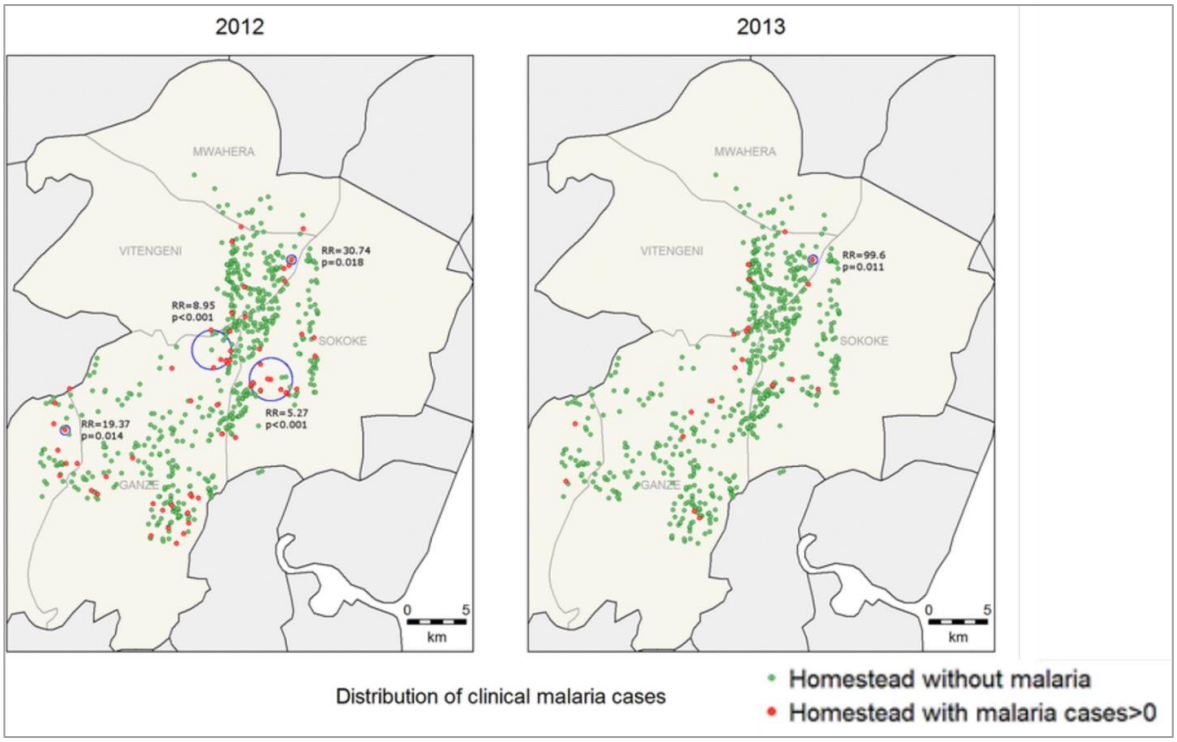

Figure 4.6*: Hotspots of clinical malaria cases in Ganze Kilifi County.*

Each blue circle *represents a statistically significant hotspot with its relative risk (RR) and p value displayed beside the circle (Kangoye et al. 2016).*

4.4.3 Trends in parameters describing hotspots and clustering of malaria cases

The median number of significant hotspots for the datasets was 1 and there was no clear trend over transmission intensity (Figure 4.7A). However, the risk ratios for primary hotspots were highest at low MPFs (Figure 4.7B) and decreased with increasing MPF. The statistical significance of hotspots was lower at very low MPFs, then increased with increasing MPF to a peak at an MPF of ~0.3, and then gradually decreased with increasing MPF after MPF >0.3 (Figure 4.7C).

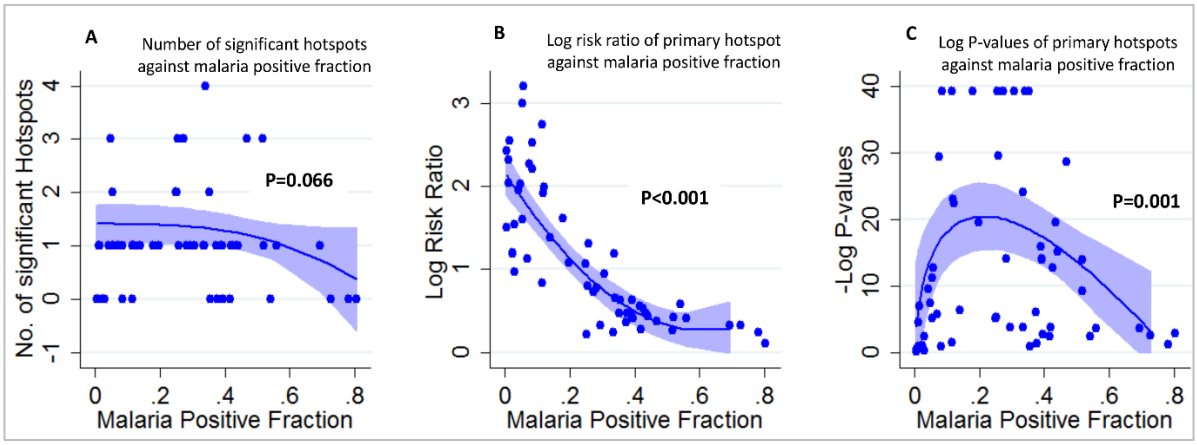

Figure 4.7: ***Hotspots of symptomatic parasitaemia.***

Panel A displays a scatter plot of the number of significant hotspots per study area against malaria positive fraction, panel B shows the Log risk ratios of malaria within the primary hotspot against the malaria positive fraction and panel C shows the –Log (P-values) of the primary hotspots against malaria positive fraction. The blue line in A, B and C show the fitted multiple fractional polynomial model predictions after adjusting for study design and the overall age of study participants. Shaded areas in panels A, B and C represent 95% CIs.

Although the average age of children in the dataset was significantly associated with the RR and p-values (Figure 4.8), analyses adjusted for average age of children in the dataset (Figure 4.7A, 4.7B and 4.7C) and analyses stratified by study design (i.e. passive vs active case detection) showed a similar trend in variation of RR over transmission intensity (Figure 4.9).

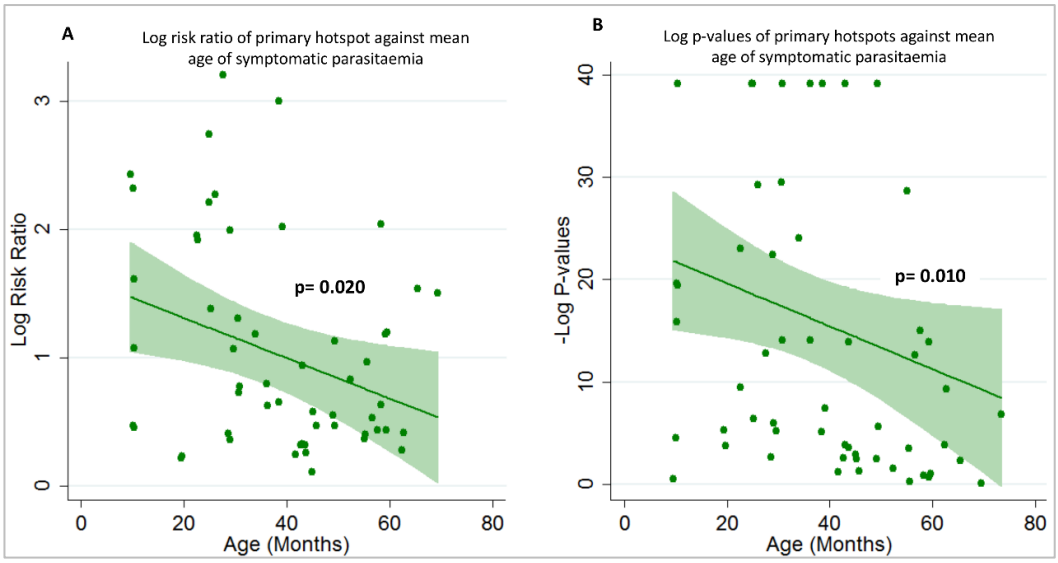

Figure 4.8: ***Trends in parameters of primary hotspots over mean age of study participants.***

Panel A shows a scatter plot of log transformed risk ratios against overall mean age. Panel B shows a scatter plot of log transformed P-values against overall mean age. The green line presents multiple fractional polynomial fits of age on MPF adjusted for the study design. Shaded areas in panels A, B and C represent 95% CIs.

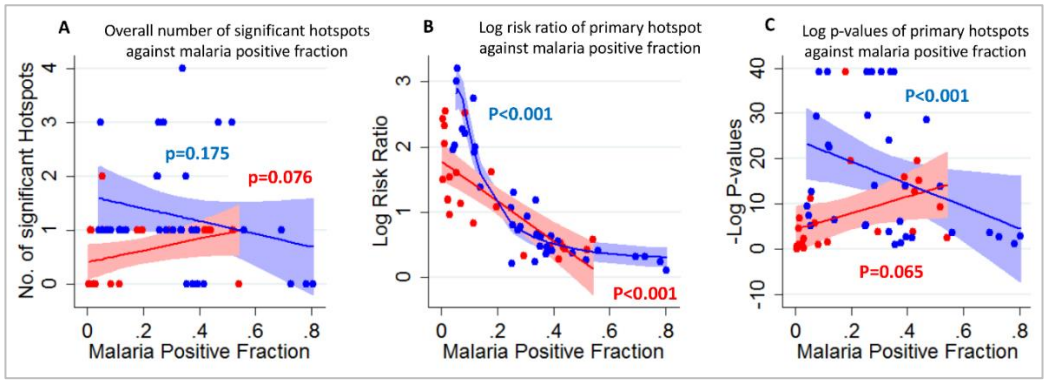

Figure 4.9: ***Summary of malaria hotspots from symptomatic parasitemia among passive (blue) and active (red) surveillance studies.***

Panel A shows a scatter plot of the no of significant hotspots against malaria positive fraction, panel B presents the Log risk ratios of malaria within the primary hotspot against the malaria positive fraction and panel C presents the –log P-values of the primary hotspots against malaria positive fraction. The blue and red lines in panels A, B and C show the fitted multiple fractional polynomial model predictions for passive and active case detection studies respectively. Shaded areas in panels A, B and C represent 95% CIs.

While there were fewer studies that included data on asymptomatic parasitaemia, a similar trend for risk ratios with increasing parasite prevalence was observed, but without a clear trend for p-values (Figure 4.10). Fractional polynomial transformations significantly improved model fits (Table 4.2).

Table 4.2 ***: Comparison between linear and the multiple fractional polynomial model fit.***
The P-value shown are derived from the log-likelihood ratio test for a nested model with a fractional polynomial over the linear fit model.

Hotspots Parameter	Figure	Best fit fractional polynomial transformations	Deviance difference	P-value	Adjusted R^2
Number of hotspots	Fig 2A	MPF__1 = MPF^3 - 0.02058	9.181	0.04	0.1589
Log risk Ratio	Fig 2B	MPF__1 = MPF - 0.27406 MPF__2 = MPF^2 - 0.07511	17.486	0.001	0.7382
- log P-value	Fig 2C	MPF__1 =MPF^0.5 - 0.5235 MPF__2 = MPF- 0.27406	11.667	0.015	0.2792

**MPF refers to Malaria Positive Fraction and MPF__1 and MPF__2 are the fractional polynomial transformations for MPF.*

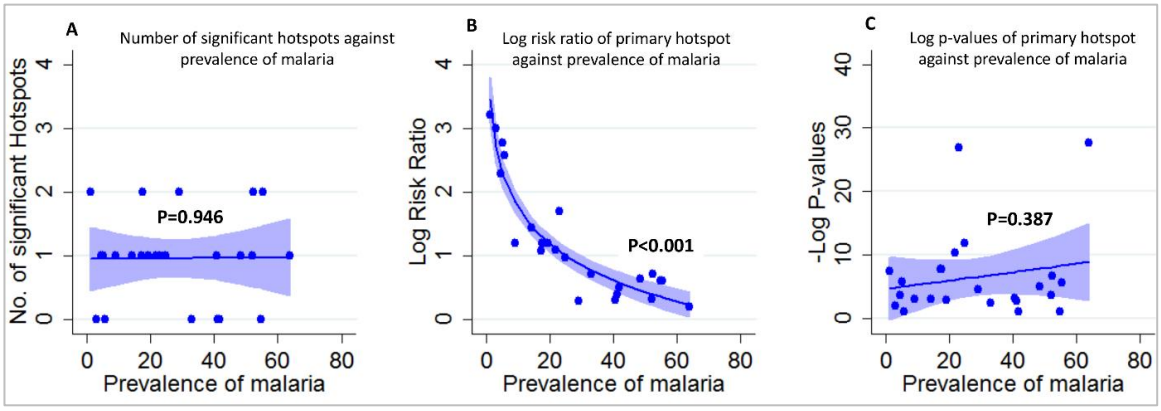

Figure 4.10: ***Hotspots of asymptomatic parasitaemia.***

Panel A displays a scatter plot of the number of significant hotspots in each study dataset against parasite prevalence, panel B presents the Log risk ratios of malaria within the primary hotspot against the parasite prevalence and panel C displays the –Log (P-values) of the primary hotspots against parasite prevalence. The blue lines in panels A, B and C show the fitted multiple fractional polynomial model predictions. Shaded areas in panels A, B and C represent 95% CIs.

Using the modelled relationship between PfPR and the reproduction number (R_0) reported by Smith et al *(Smith et al. 2006)*, I determined the ratios of R_0 inside and outside hotspots, and plotted these against PfPR (Figure 4.11). The ratio of R_0 inside to R_0 outside rose steeply below a parasite prevalence of 10%.

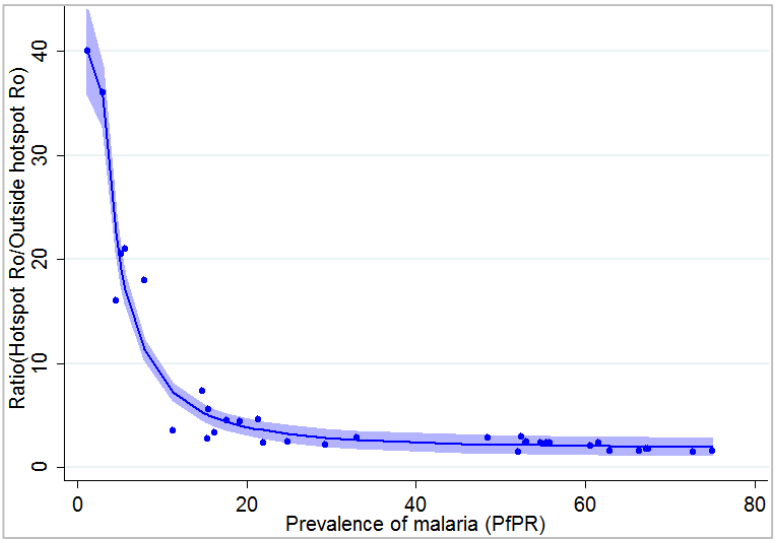

Figure 4.11: ***Scatter plot of the ratio of log transformed R0 inside to outside the hotspot plotted against parasite prevalence.***

The blue line shows the fitted multiple fractional polynomial model predictions and the shaded areas represents 95% CIs.

4.4.4 Spatial autocorrelation

Ripley's difference in k-function (i.e. the D-function) indicated significant spatial structure in many but not all sites (Figure 4.12). As seen with hotspots, the proportion of sites that had significant spatial structure increased from the lowest MPFs to a peak at MPFs of 0.15 - 0.45, and then declined at higher MPFs. This trend was consistent at the various spatial scales examined (Figure 4.12). The magnitude of the D function decreased with increasing MPF and was consistent at various spatial scales (Figure 4.13).

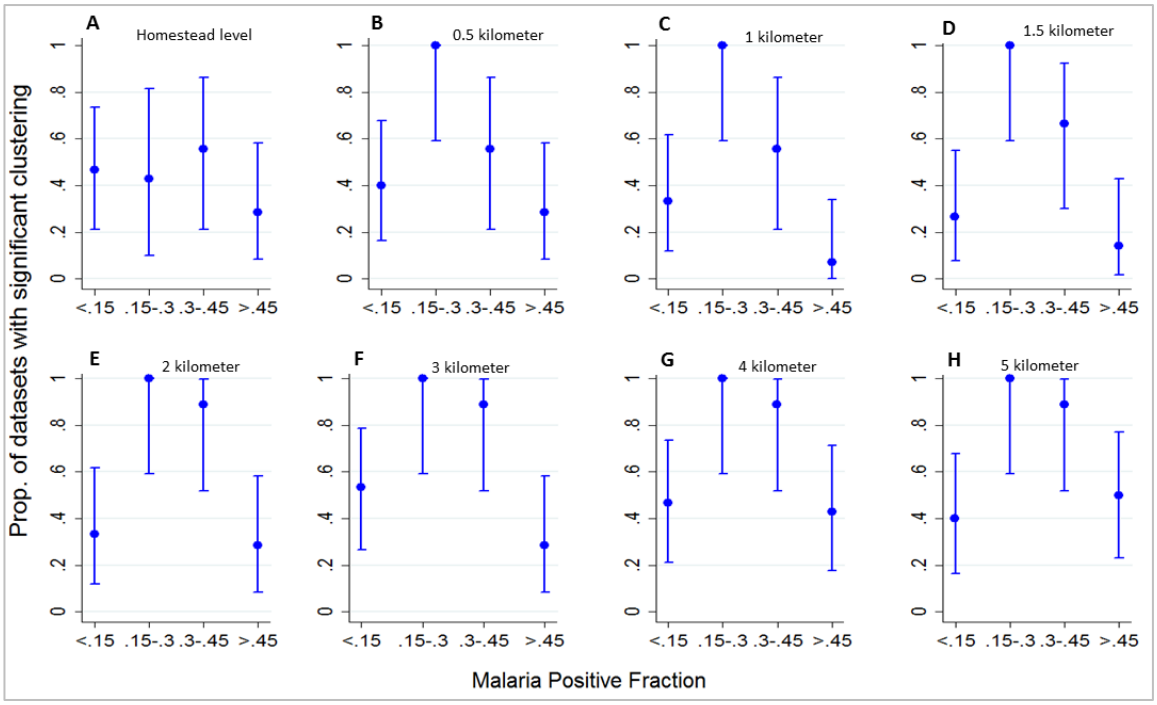

Figure 4.12: ***Clustering of malaria transmission.***

Panels A, B, C, D, E, F, G, and H shows the proportion of datasets with significant clustering at homestead level, 0·5, 1, 1·5, 2, 3, 4, and 5-kilometer level respectively, against malaria positive fraction.

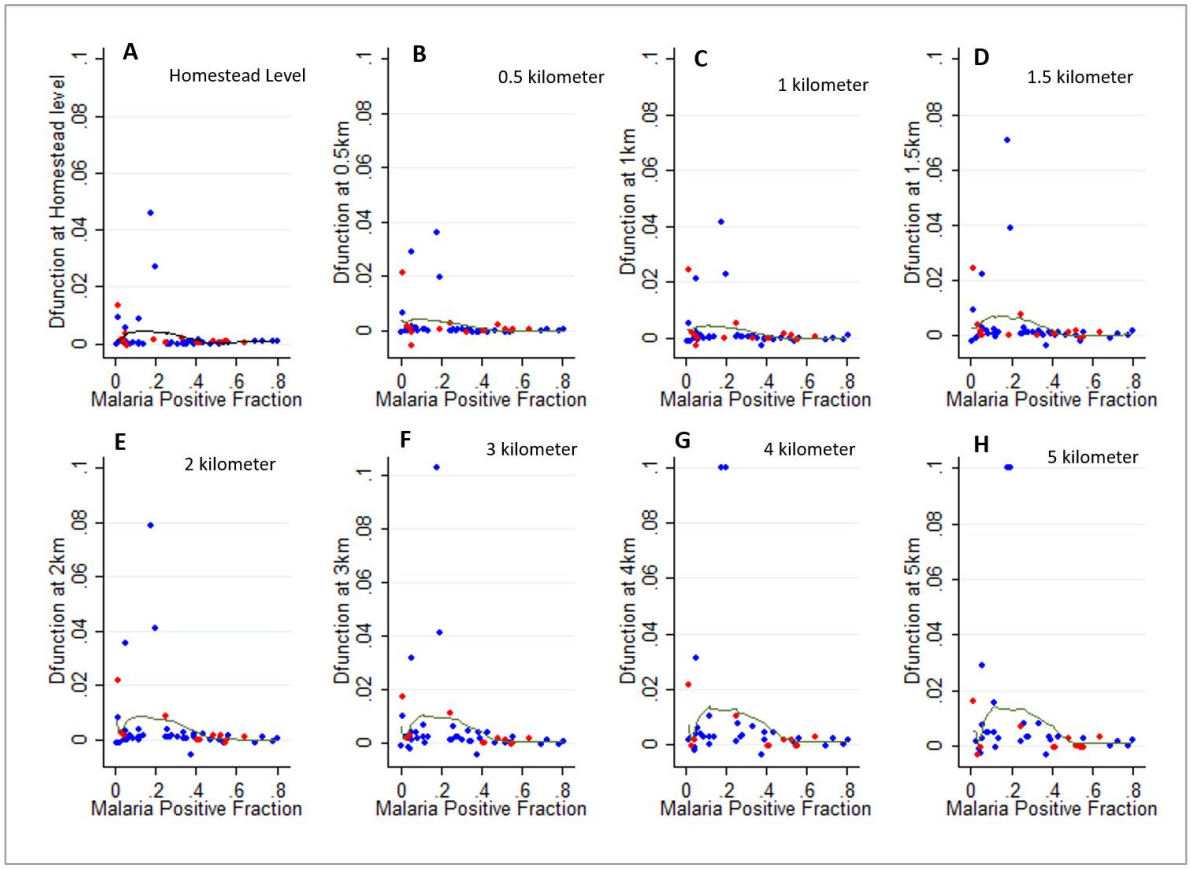

Figure 4.13: Difference in K-functions for cases and controls (D-function) against malaria positive fraction.

Panels A, B, C, D, E, F, G, and H show the D-function at homestead level, 0·5, 1, 1·5, 2, 3, 4 and 5 kilometer distances for each dataset. The blue dots represent symptomatic parasitaemia datasets while red represents asymptomatic parasitaemia datasets.

4.4.5 Temporal trends

Overall, the spatial distribution of asymptomatic parasitaemia showed modest temporal stability in the Asembo and Burkina Faso sites (Table 4.3). On the other hand, the spatial distribution of febrile malaria was predictive of febrile malaria over one and two years in Uganda, but not in the other sites.

Table 4.3*: Association between distribution of MPF within grids (size= 2x2 km2) over time interval*

(Asembo Bay, Kenya (Hawley et al. 2003), sub-counties of Uganda (Kamya et al. 2015) and Afigya-Sekyere Ghana (Kreuels et al. 2008)) in years and over consecutive cross-sectional surveys conducted over a span of one year (Saponé district, Burkina Faso (Tiono et al. 2013)).

		Febrile Malaria		Asymptomatic Parasitaemia	
Study Site	Interval between cluster (year)	Correlation (95%CI)	P-value	Correlation (95%CI)	P-value
Asembo Bay	1	-0.09 (-0.26 - 0.09)	0.3072	0.23 (0.08 - 0.36)	0.003
	2	0.14 (-0.04 - 0.31)	0.1245	0.16 (0.01 - 0.31)	0.0433
	3	0.16 (-0.08 - 0.38)	0.1873	0.02(-0.18 - 0.22)	0.8512
	4	0.45 (0.11 - 0.70)	0.0124	0.21 (-0.12 - 0.49)	0.2041
	5	0.06 (-0.32 - 0.43)	0.7726	0.45 (-0.13 - 0.80)	0.1226
Burkina Faso	Interval between cluster (Survey)				
	1	-0.07 (-0.21 - 0.08)	0.3667	0.24 (0.10 - 0.36)	<0.001
	2	0.06 (-0.13 - 0.24)	0.5359	-0.09 (-0.25 - 0.08)	0.293
	3	0.27 (0.01 - 0.50)	0.0457	0.34 (0.11 - 0.53)	0.0043
Uganda	Interval between cluster (year)				
	1	0.39 (0.27 - 0.50)	<0.001	_	_
	2	0.29 (0.13 - 0.44)	0.001	_	_
	3	0.19 (-0.06 - 0.41)	0.1332	_	_
Ghana	Interval between cluster (year)				
	1	0.26 (-0.03 - 0.51)	0.0756	_	_
	2	0.30 (-0.14 - 0.64)	0.1757	_	_

4.4.6 Average age of symptomatic malaria episodes and correlations with MPF

Spearman's rank correlation coefficients of MPF against average age of symptomatic children with malaria at various spatial scales (i.e. $1km^2$, $2km^2$ and $4km^2$ grids) were negative in most study datasets. This suggests that patches of greater exposure to malaria (i.e. high MPF) were associated with younger children presenting with malaria parasites in their blood and vice versa

(Figure 4.14, Figure 4.15 and Figure 4.16). The pooled estimates increased with increasing grid size agreeing with previous findings *(Bejon et al. 2014).*

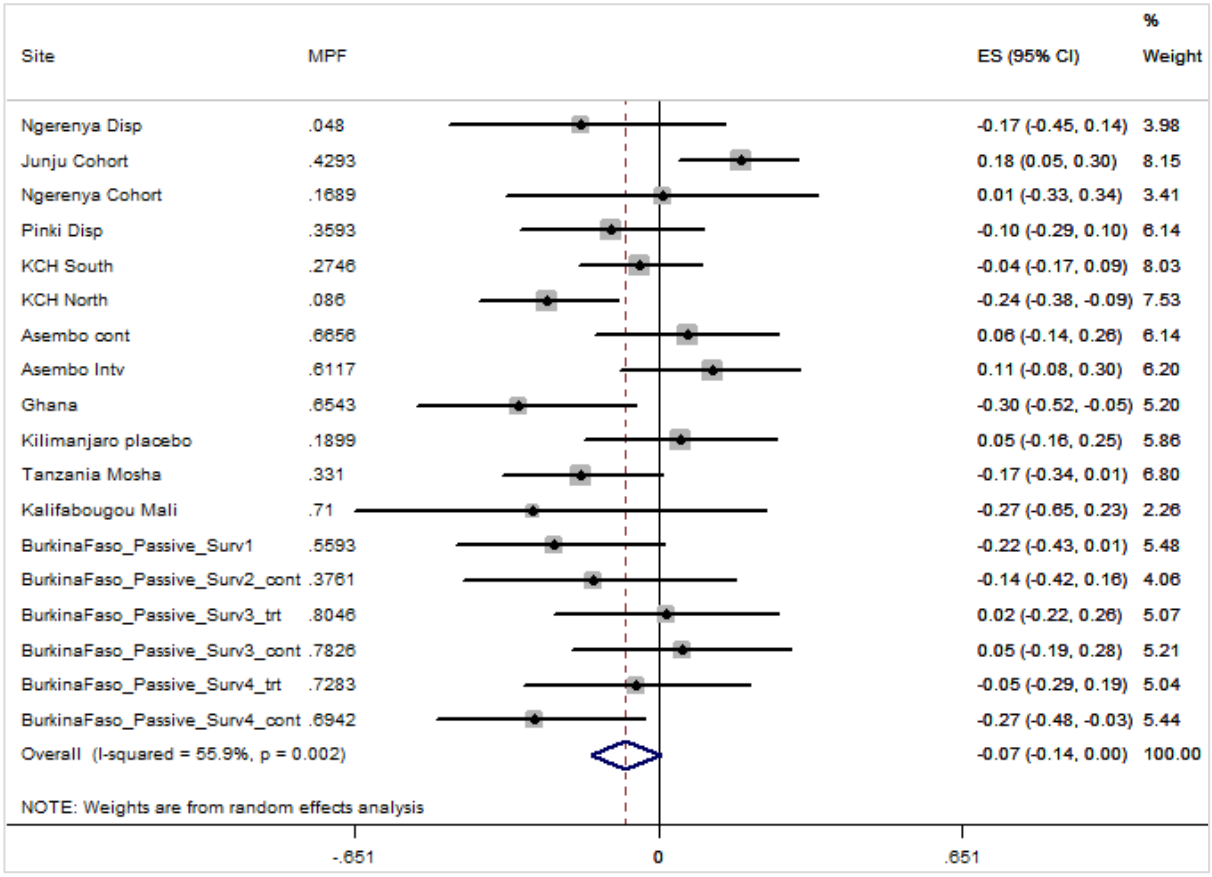

Figure 4.14: ***Forest plot showing the correlation (Rho) between MPF and age of symptomatic malaria at a 1x1 km grid size.***

The pooled estimate from the random-effects meta-analysis is labeled "Overall".

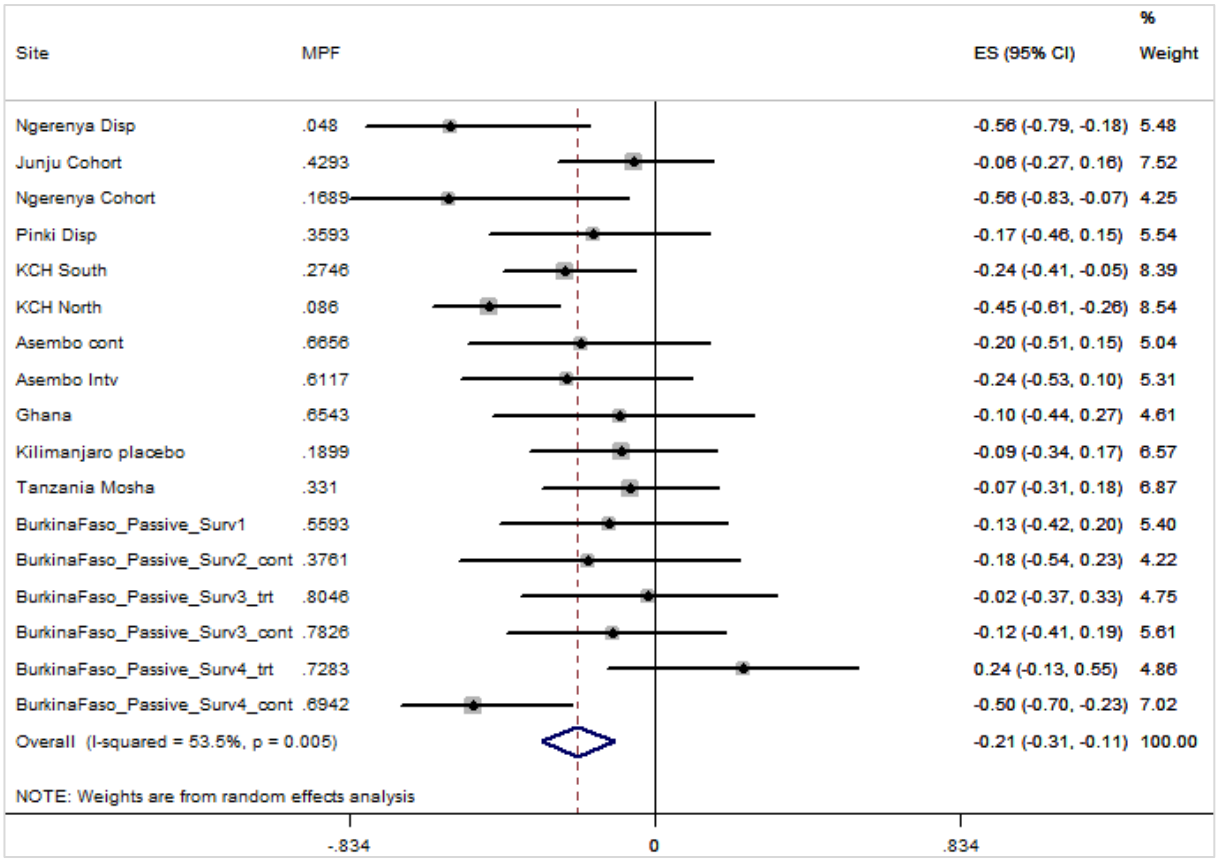

Figure 4.15: ***Forest plot showing the correlation (Rho) between MPF and age of symptomatic malaria at a 2x2 km grid size.***

The pooled estimate from the random-effects meta-analysis is labeled "Overall".

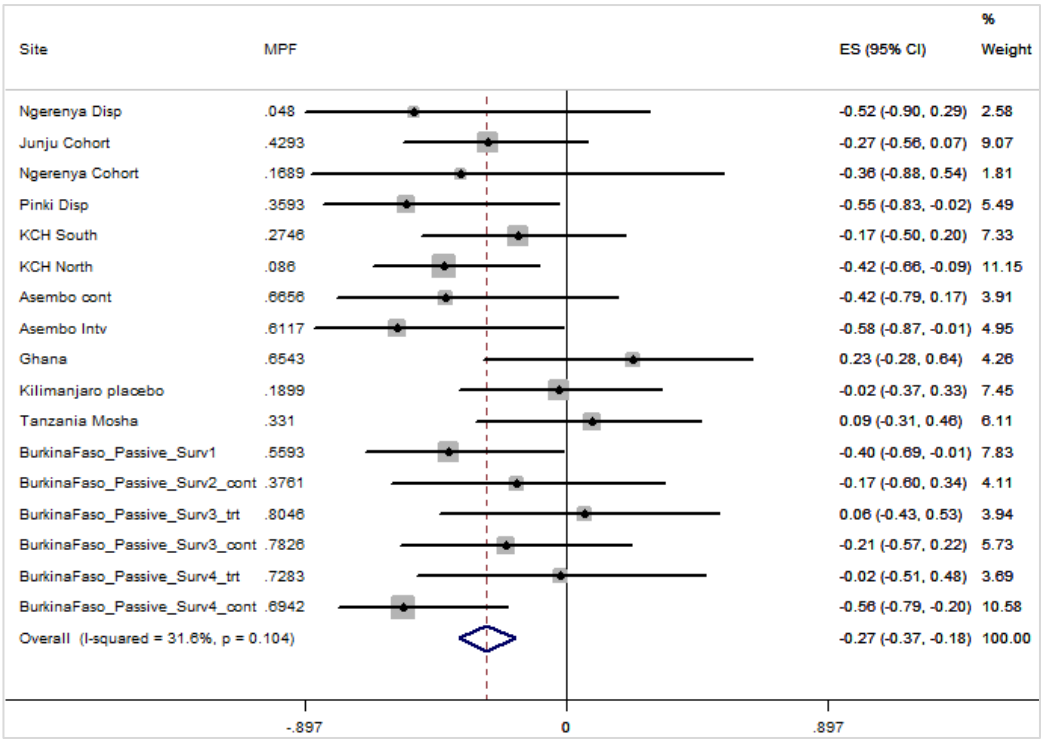

Figure 4.16: ***Forest plot showing the correlation (Rho) between MPF and age of symptomatic malaria at a 4x4 km grid size.***

The pooled estimate from the random-effects meta-analysis is labeled "Overall".

Significant moderate heterogeneity was observed between studies at the 1x1 and 2x2 Km square spatial grid but not at a 4x4 grid size. The funnel plot resembled a symmetric funnel for the 1x1 km grid (Figure 4.17 A) but not for the 2x2 and 4x4 km grid sizes (Figure 4.17 B and C). However, it should be noted that some datasets included in Figure 4.17 A, could not be included in Figure 4.17 B and C due to the size of the study area not permitting >10 grid sizes that could enable the computation of correlations when lager grids were used.

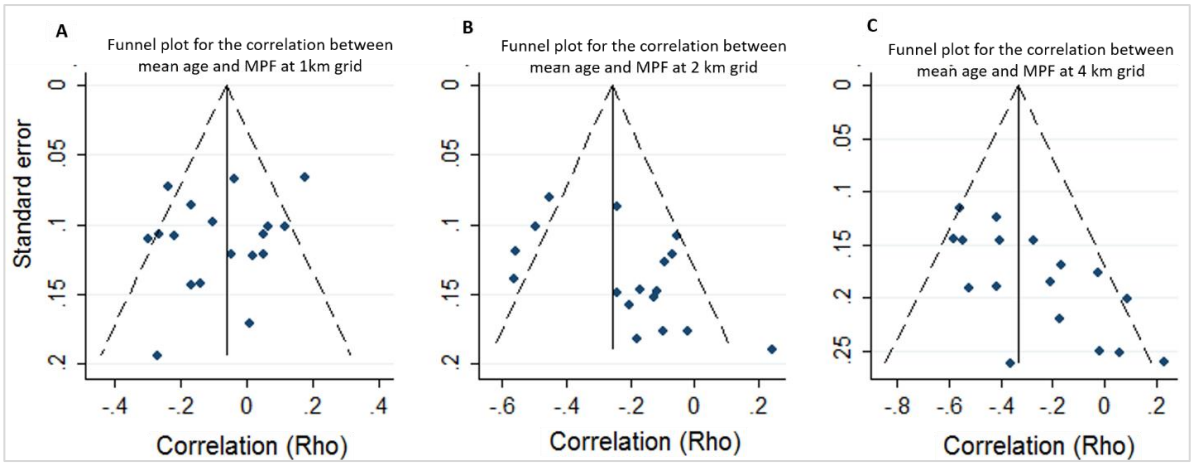

Figure 4.17: ***Funnel plot with pseudo 95% confidence limits.***

Panels A, B and C show the funnel plots of individual study standard errors plotted against the correlation between the mean age of clinical malaria and MPF at prespecified spatial grids.

These negative associations tended to be more marked where the average MPF at the site was low, and this trend was statistically significant when the correlations were measured using $2km^2$ grids (i.e. p=0.04) but not at $1km^2$ or $4km^2$ grids (Figure 4.18 A, B and C). The pooled correlation between MPF and slide positive age for 1x1, 2x2 and 4x4 kilometer spatial resolution was -0.07 (95% CI -0.14 to 0.00), -0.21 (95% CI -0.31 to -0.11) and -0.27 (95% CI -0.37 to -0.18) respectively and were in the same direction. The test of heterogeneity between studies was I^2=55.9%, p=0.002; I^2=53.5%, p=0.005; and I^2=31.6%, p=0.104 respectively.

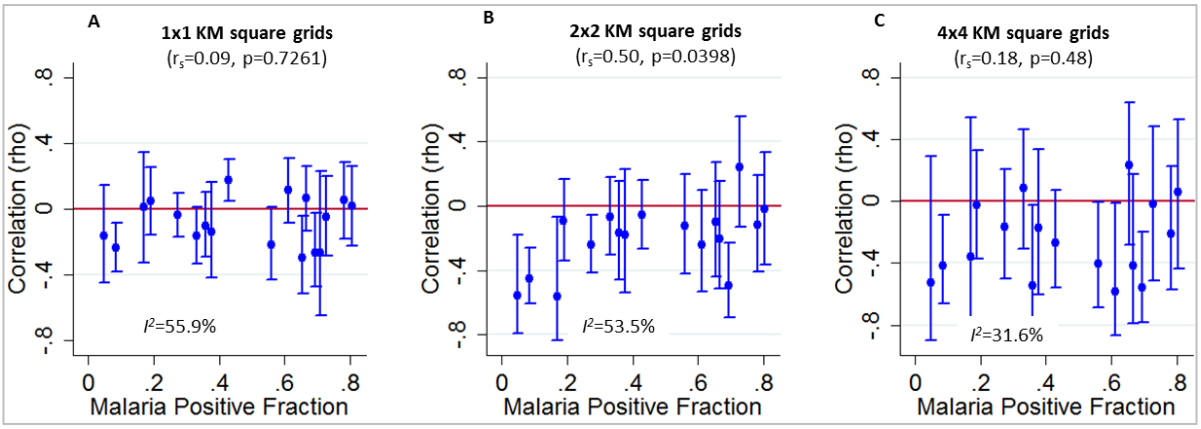

Figure 4.18: ***Fine-scale geographical correlation of mean age (months) against malaria positive fraction (MPF) for each study dataset plotted against overall study MPFs (as a proxy for transmission intensity).***

Panels A, B and C show 1x1, 2x2, and 4x4 km^2 grid respectively. The test of heterogeneity between studies was I^2=55·9%, p=0·002; I^2=53·5%, p=0·005 and I^2=31·6%, p=0·104 respectively.

Furthermore, I observed significant spatial autocorrelation for the age of symptomatic malaria episodes at most sites (Figure 4.19), suggesting that there are focal areas where older individuals tend to be seen with symptomatic malaria, and conversely focal areas where younger individuals tend to be seen with symptomatic malaria.

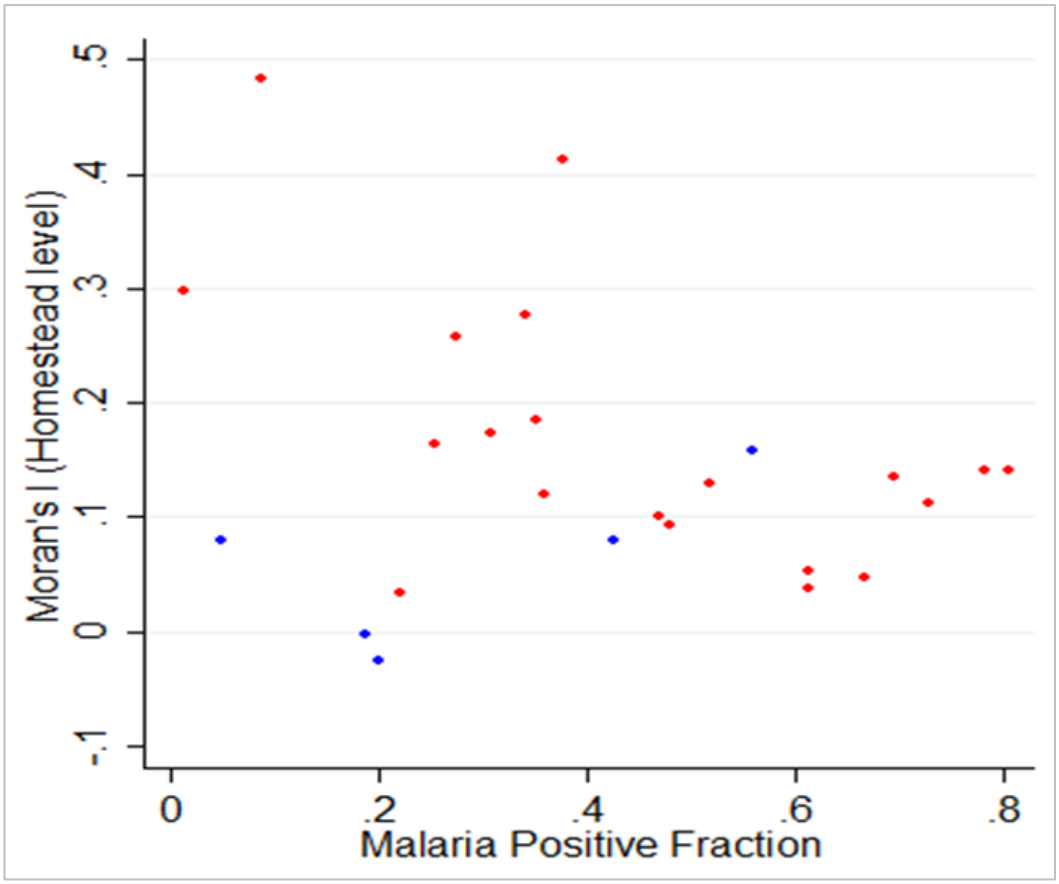

Figure 4.19: ***Homestead level spatial autocorrelation of age in months for symptomatic individuals for the various studies.***

Red dots show significant autocorrelation while blue dots show non-significant spatial autocorrelation.

4.5 Discussion

This chapter describes fine-scale spatial heterogeneity of *P. falciparum* malaria cases from studies conducted at 19 different sites experiencing varying transmission intensities in seven sub-Saharan African countries (Figure 4.1). The RR of the primary hotspots increased with falling MPF. The strength of evidence (as measured by p-values) increased from low MPFs to moderate MPFs and then declined towards high MPFs. Taking these findings on variation in degree of heterogeneity and on statistical significance of heterogeneity together, I conclude that spatial heterogeneity becomes gradually more marked as transmission intensity falls, albeit with statistical significance becoming weaker at very low transmission intensity because of reduced power due to small numbers of malaria cases. It may therefore be appropriate for malaria control programmes to target hotspots at low transmission intensity despite apparently modest statistical significance. The decline in the degree of spatial heterogeneity towards high MPF may be due to either more even distribution of transmission intensity per se, or to saturation in the metric used to quantify malaria exposure (i.e. the MPF).

Similar findings were seen for generalized measures of spatial autocorrelation where the degree of spatial autocorrelation (D-functions) is shown to increase as MPF falls (Figure 4.13) with significance testing showing a peak when MPF is within 0.15-0.45 range (Figure 4.12).

Hotspots of stable asymptomatic parasitaemia have previously been described in Kilifi, Kenya *(Bejon et al. 2010)*. I could quantify the temporal stability of the spatial distribution in 4 datasets outside Kilifi (where these studies have previously been conducted *(Hawley et al. 2003, Kreuels et al. 2008, Tiono et al. 2013)*). This showed temporal instability, however, with 4 datasets I was unable to examine trends in stability across sites. An indirect approach to examining temporal stability is to look for evidence of spatial variation in clinical immunity. Micro-

variation of malaria transmission is likely to lead to variations in the rate and degree of acquisition of clinical immunity if the micro-variation is sufficiently stable. Children acquire immunity against symptomatic malaria following repeated exposure. At high transmission intensity, children acquire immunity rapidly due to intense exposure when they are young and hence do not present with symptomatic malaria when they are older. On the other hand, at low transmission, children acquire immunity slowly and are more likely to present with symptomatic malaria when they are older *(Snow et al. 1997).* As might therefore be predicted, I observed a negative correlation between MPF and age of symptomatic malaria keeping with previously reported findings *(Bejon et al. 2014, Mogeni et al. 2016),* and is taken to imply that immunity is acquired more rapidly with greater exposure to malaria, leading to a lower average age of symptomatic malaria episodes. Furthermore, there were positive autocorrelations (i.e. significant values of Moran's I) in the age of children with symptomatic malaria, again suggesting that micro-variation of transmission intensity may have led to variation in the degree of acquisition of clinical immunity.

In the 4 datasets with longitudinal data, the temporal stability of the distribution of clinical malaria was lower than that seen in previous analyses in Kilifi and the highlands of Western Kenya *(Ernst et al. 2006, Bejon et al. 2010).* Furthermore, I identified substantial heterogeneity in the correlations between MPF and age of symptomatic malaria. Taking these findings together, I conclude that temporal stability of hotspots is not a reproducible feature of malaria transmission. I did not identify a strong trend of greater spatial stability at any range of MPF (Figure 4.18).

Mathematical models suggest that targeting control interventions on hotspots results in a more marked decline in malaria compared to untargeted interventions with an equal amount of

resources *(Bousema et al. 2012b)*. To implement such a strategy requires the accurate identification of hotspots and our data suggest that hotspots may not be temporally stable, and may be more difficult to accurately identify at high transmission. A previous attempt at targeting hotspots of malaria transmission in Rachuonyo – an area of moderate transmission intensity in western Kenya - achieved modest reductions in transmission inside the targeted hotspots, but no lasting reductions outside the targeted hotspots in a cluster-randomized control trial *(Bousema et al. 2016)*. The authors suggested that the limited impact was at least partly explained by challenges in defining the geographical boundaries of transmission hotspots *(Bousema et al. 2016)*; our findings on temporal instability of hotspots would confirm difficulties in defining hotspots.

Study limitations include the use of data collected using microscopy which is of limited sensitivity for parasitaemia. PCR has been shown to be more sensitive for parasitaemia, particularly in low transmission regions *(Okell et al. 2012)*. This is unlikely to bias studies based on febrile malaria episodes since symptomatic malaria individuals usually have parasite densities well above the detection threshold for microscopy. However, submicroscopic infections among studies of asymptomatic parasitaemia may influence the stability of hotspots. Most studies included applied a threshold parasitaemia to define febrile malaria. The threshold reduces the likelihood that cases of asymptomatic parasitaemia with co-incident fever are non-specifically included in febrile malaria cases *(Mwangi et al. 2005, Bejon et al. 2007)*.

The modifiable areal unit problem may lead to bias when an arbitrary grid size is used to aggregate data. I mitigated this problem by conducting a sensitivity analysis using grids with varying sizes (i.e. 1km, 2km and 4km squares). A further limitation is that detection of febrile malaria is influenced by study design, sample size and targeted age group, which was not

standardized across studies. However, I observed similar results even after adjusting for these potential confounders, and identified similar results for studies of febrile malaria and of asymptomatic parasitaemia.

Most of the studies included were conducted in relatively high to moderate transmission settings or in low transmission settings following recent reductions in transmission. Areas that have historically experienced low transmission may be under-represented, and furthermore study sites were clustered within West and East Africa without representation of Central and Southern Africa. Only researchers interested in malaria hotspots were targeted to contribute data used in this study thus presenting a potential source of bias.

4.6 Conclusions

I found geographical micro-variation in malaria transmission within sites from across sub-Saharan Africa at a variety of transmission intensities. Micro-variation was greater in low transmission settings, albeit with less statistical power to detect it where cases of malaria are few. The temporal instability of hotspots and the difficulties in defining hotspots (especially in higher transmission settings) will be a challenge to targeted control. However, given the predictability with which hotspots occur when transmission intensity is low (e.g. PfPR<10%), malaria control programmes should have a low threshold for responding to apparent clustering of cases. The ratio of R_0 inside to R_0 outside rose steeply below a parasite prevalence of 10%, suggesting that the potential to interrupt transmission by targeting hotspots increases below this prevalence. Many sub-Saharan African countries currently contend with high malaria transmission and based on recent evidence *(Bousema et al. 2016)*, are unlikely to benefit

significantly from targeted control. However, some countries have witnessed substantial declines (such as, Zanzibar *(Bhattarai et al. 2007)*, Swaziland *(Kunene et al. 2011)* among others) that warrant the implementation of targeted control to achieve elimination. Our data predicts that hotspots will be a marked feature of transmission in such settings.

Chapter Five

5 Detecting malaria hotspots: a comparison of RDT, microscopy and polymerase chain reaction

5.1 Introduction

The efficacy of targeting hotspots of malaria transmission is likely to be higher if hotspots are detected accurately and their stability determined. I have shown in chapter 4 that the stability of hotspots may not be a generalizable property of malaria transmission. However, this potentially varies according to the diagnostic tools used to assess parasitaemia. For instance, the lower densities detectable by PCR could lead to greater temporal stability of the heterogeneity detected.

5.2 Objectives

- To quantify the extent to which hotspots of malaria transmission detected by RDT and microscopy overlap geographically with those detected by PCR.
- To examine the variability in temporal stability of hotspots identified by the three diagnostic tools.

5.3 Method

5.3.1 Ethics statement

Approval for human participation in cross-sectional surveys was given by the Kenya Medical Research Institute Ethics Research Committee. Prior to any study procedure, written informed consent was obtained from all individuals participating in the surveys or, where appropriate, guardian/ parental consent was sought for children. The studies were conducted per the principles of the declaration of Helsinki.

5.3.2 Study sites

I analysed data from annual cross-sectional surveys conducted within three separate cohort studies in Kilifi County on the Kenyan coast. The Junju cohort is located within the southern part of the Kilifi Health and Demographic Surveillance System (KHDSS) area (Figure 5.1) *(Scott et al. 2012)* and experiences perennially higher malaria transmission intensity *(Mogeni et al. 2016)* compared to the Ngerenya and Ganze cohorts which are located to the north.

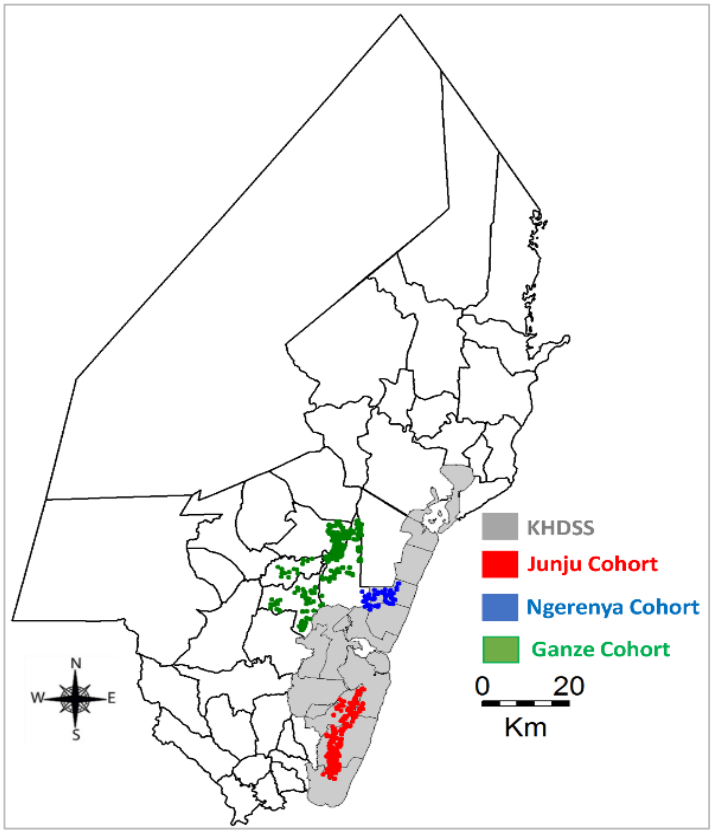

Figure 5.1*: Map of Kilifi county showing the KHDSS area (shaded grey), and the homesteads where the studies were conducted.*

Annual surveillance of asymptomatic malaria in these cohorts has been described in detail elsewhere *(Bejon et al. 2010, Kangoye et al. 2016)*. Briefly, in the Junju and Ngerenya cohorts, cross-sectional surveys were undertaken annually between 2007 and 2016 in Junju, and between 2007 and 2014 in Ngerenya *(Bejon et al. 2010)*. Surveys took place in April and May of each

year, just before the rainy season and all individuals recruited to the study cohorts were invited to participate by providing a blood sample for malaria diagnosis. In Ganze, two cross-sectional surveys were conducted, first, between July and September 2012, and second, between May and July 2013 *(Kangoye et al. 2016)*. Global positioning system coordinates were linked to every homestead in each cohort.

5.3.3 Laboratory procedures

Presence of malaria parasites was examined using RDTs, microscopy and PCR by trained laboratory technicians and standardized across the sites. Blood samples were obtained from all children under 15 years of age whose consent to participate in the study had been obtained *(Bejon et al. 2010, Kangoye et al. 2016)*. Children with fever (that is, axillary temperature >37.5^{o}C) were referred for immediate assessment and treatment and not included in the survey data. Each sample collected was assessed for parasitaemia using RDT, microscopy and PCR in all sites.

RDT analysis - The RDT used for the study is CareStart Malaria test manufactured by AccessBio Inc. RDTs detect the presence of *P. falciparum* histidine-rich protein 2 (HRP2) and *P. falciparum* lactate dehydrogenase (PLDH) in the blood. RDT stocks were stored in an air-conditioned room with monitored temperature and humidity. Quality assurance for the stored test kits was conducted regularly before use by trained laboratory technicians.

Microscopy analysis - Thick and thin blood smears were Giemsa stained and examined using light microscopy at 1,000x magnification for malaria parasites and malaria species respectively. Malaria infection and parasite counts by microscopy were determined independently by two readers and discordant readings resolved by a third reader. The number of parasites/200 white blood cells (WBCs) was counted and parasite density per microliter of blood calculated using

an average count of 8000 WBCs/µl of blood as described elsewhere *(Mwangi et al. 2005)* and reported by species (that is; *P. falciparum*, *P. malariae* and *P. ovale*).

PCR analysis - DNA was first extracted from 30 µl of whole blood using QIAxtractor machine (QIAGEN, Hilden, Germany). The DNA was eluted in 100 µl from which 5 µl of DNA was amplified by quantitative PCR. This was done using a TaqMan® assay for the *P. falciparum* multicopy 18S ribosomal RNA genes as described elsewhere *(Hermsen et al. 2001)*, except that a modified probe (5′-FAM-AACAATTGGAGGGCAAG-NFQ-MGB-3′) as described elsewhere *(Sheehy et al. 2012)* was used. An Applied Biosystems 7500 Real-Time PCR System with quantification by Applied Biosystems 7500 software v2.0.6 was used. Samples were analysed in singlet wells. Three negative control wells and 7 serial dilutions of DNA extracted from *in vitro* parasite cultures were included as standards on each plate in triplicate *(Ogwang et al. 2015)*. Plates failing quality control standards were repeated. The lower limit of accurate quantification of this method is 10 parasites/mL within the PCR elute, and by assessing $1/20^{th}$ of 30 µl with a gene target present on 3 chromosomes have a theoretical limitation of 4.5 parasite per µl of whole blood, compared with a sensitivity of 50 parasites per µl for thick blood films.

Laboratory technologists assessing malaria using each given diagnostic tool were blinded to the results of the other diagnostic tools. RDT, microscopy and PCR standards were monitored through a quality assurance scheme that included comprehensive training during induction and at regular intervals during the study period. Microscopy quality assurance was evaluated using external quality control slides.

5.3.4 Geographical cluster analysis

Individuals who had complete data on RDT, PCR and microscopy were included in the analysis. Hotspots are defined as geographical areas experiencing significantly higher prevalence of asymptomatic parasitaemia than would be expected by chance. In this study, I assess chance using the spatial scan statistic *(Kulldorff 1997)* through the Bernoulli model in SaTScan™ software v9.4.1 as described in the methods section. Local clusters of RDT, PCR and microscopy data were assessed separately and the differences in parameters (i.e. risk ratios (RR), hotspots radius and P-values) compared.

5.3.5 Temporal variation in malaria transmission

Parasite prevalence was computed by imposing spatial grids on the data and collapsing to the mean prevalence within each cell of the grid. This was done with grids of variable sizes 0.5x0.5km, 1x1km and 2x2km grid squares, selected *a priori* to allow for a sensitivity analysis that would examine the potential bias resulting from the modifiable areal unit problem, and repeated by year. The association between parasite prevalence by PCR and by microscopy or RDT was assessed for the various grid sizes. Furthermore, the stability of spatial heterogeneity for RDT, PCR and microscopy datasets was assessed by examining parasite prevalence within grids separated in time using the Spearman's rank correlation coefficient.

The degree to which hotspots overlap was defined as the fraction of homesteads within the intersection of hotspots detected by PCR and microscopy or RDT divided by the total number of homesteads within the hotspots. Only homesteads within primary hotspots (most likely cluster regardless of significance) and any other statistically significant secondary clusters were included in the computations.

Hotspots of malaria transmission were mapped on Google Map extracts in R version 3.3.1 *(Team 2016)*. Graphs were generated, Cohens' Kappa statistic was computed and correlation analyses using Stata version 12 (StataCorp, Texas).

5.4 Results

A total of 8581 study participants were surveyed in the three study sites. There was a positive correlation between *P. falciparum* parasite density measured by PCR and by microscopy among those testing positive (Figure 5.2a) (r=0.72, p<0.001, and a strong association between detection by PCR and detection by microscopy (Table 5.1, kappa=0.6159, p<0.001).

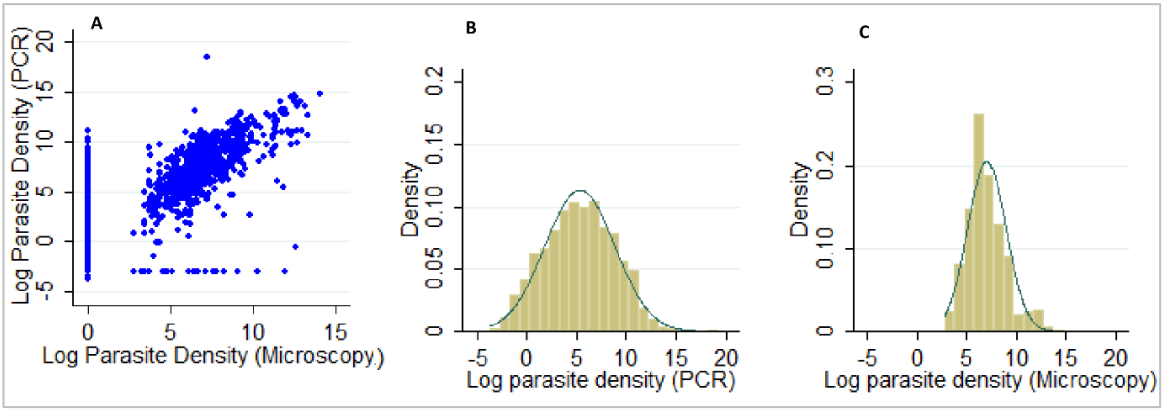

Figure 5.2*: Distribution of parasite densities.*

Panel A shows a scatter plot of Log-transformed parasite/µl densities detected by microscopy and PCR (PCR negative test results were assigned an arbitrary value of 0.05 parasite/µl, while microscopy negative test results were assigned an arbitrary value of 1 parasites/µl before log transformation to allow complete data presentation for samples that were either positive by PCR or microscopy). Panels B and C show histograms of log-transformed PCR and microscopy parasites/µl densities respectively, against normal distribution functions.

Table 5.1: *Number of samples that were malaria parasite positive by microscopy and PCR*

Site	N	Malaria test result			
		Both PCR and Microscopy n(%)	PCR only n(%)	Microscopy only n(%)	Negative by PCR and microscopy n(%)
Junju	4910	790 (16.1)	688 (14.0)	22 (0.45)	3410 (69.45)
Ngerenya	2406	5 (0.21)	44 (1.83)	0 (0)	2357(97.96)
Ganze	1265	12 (0.95)	62 (4.90)	1(0.08)	1190(94.07)
Total	8581	807 (9.40)	794 (9.25)	23 (0.26)	6957(81.07)

Parasite densities by PCR and microscopy were log-normally distributed (Figure 5.2b and 5.2c). The geometric mean PCR densities (of positive samples) were lowest in Ngerenya 11.79 parasites/µl (95%CI 3.68-37.76 parasites/µl) and highest in Junju 220.02 parasites/µl (95%CI 184.17-262.85 parasites/µl).

5.4.1 Hotspots of malaria transmission

Malaria species were only examined by microscopy. Overall, the prevalence of malaria by species in the three sites were 9.67% (830/8581 films), 0.16% (13/8004 films), 0.60% (48/8014 films) and 0% (0/8014 films) for *P. falciparum, P. ovale, P. malariae* and *P. vivax* respectively. *P. ovale* and *P. malariae* were only detected in the moderate transmission site (Junju) and not in either of the low transmission sites. No *P. vivax* case was reported in any of the sites.

In pooled data analysis from Junju, I identified 2 statistically significant hotspots of *P. falciparum* (radius=1.75 km, RR=2.69, p<0.001) and (radius=1.07 km, RR=2.87, p<0.001). I identified one significant primary hotspot of *P. malariae* (radius=0.053 km, RR=10.41, p=0.003) and a borderline significant secondary hotspot (radius=0 km, RR=9.33, p=0.065) and a non-significant hotspots of *P. ovale* (radius=1.76 km, RR=6.3, p=0.439). The hotspots of *P. falciparum, P. malariae* and *P. ovale* overlapped geographically (Figure 5.3). Further analysis is restricted to *P. falciparum.*

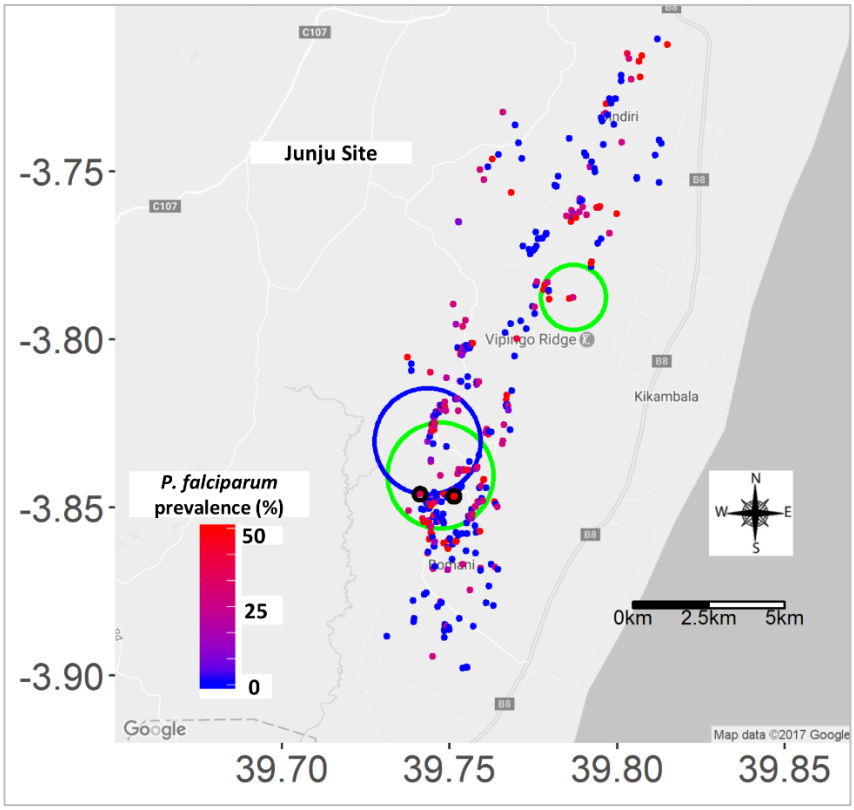

Figure 5.3*: Hotspots of malaria transmission by species.*

Green (significant primary and secondary hotspots), blue (primary non-significant hotspot) and the black (significant primary and secondary hotspots) circles represent hotspots of P. falciparum, P. ovale and P. malariae respectively as detected by microscopy.

P. falciparum was detected by PCR, RDT and microscopy. Significant hotspots of malaria transmission by the three diagnostic tools were observed in the Junju and Ganze sites. However, hotspots of malaria transmission in Ngerenya were statistically significant only when measured by PCR and RDT, and not statistically significant when measured by microscopy (Table 5.2). Overall (pooled data analysis across all years of monitoring), the degree of overlap between hotspots detected by PCR and those detected by microscopy was 100% in Junju, but less overlap was noted when hotspots were examined by year (Table 5.2 and Figure 5.4).

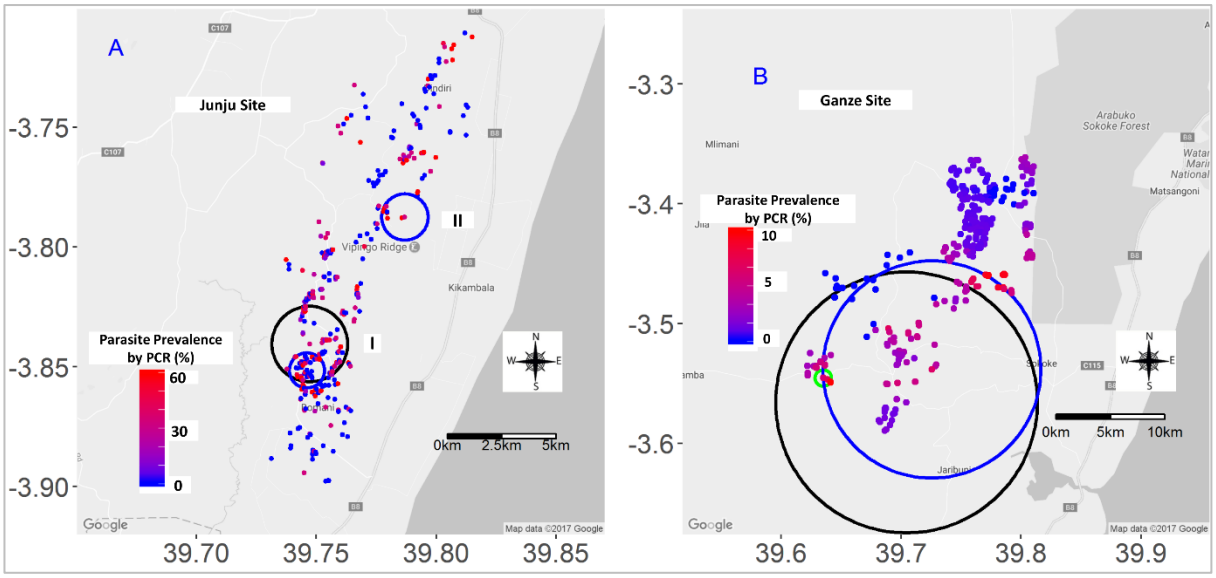

Figure 5.4*: Hotspots of malaria transmission by diagnostic tool.*

Panels A and B show the Junju (moderate transmission) and Ganze (low transmission) cohorts respectively. In Junju, there was complete overlap between PCR (black circles) and microscopy (green circles) but partially by RDT (blue) for the primary hotspot (I). However, for the three diagnostic tools used, there was complete overlap in the significant secondary hotspots (II). In Ganze, the hotspot detected by microscopy (green circle) was within the hotspots detected by PCR (black circle) and at the border with RDT (blue circle).

However, in the Junju site, there was partial overlap of PCR and RDTs' primary hotspots (45.9%), but complete overlap for the significant secondary hotspots (Table 5.2). Overall, overlap in hotspots detected in Ganze and Ngerenya sites were inconsistent. The risk ratios for microscopy hotspots were consistently larger than those measured by PCR.

Table 5.2: ***Properties of malaria hotspots and degree of homestead overlap between hotspots detected by PCR, microscopy and RDT.***

		PCR			Microscopy			RDT			Degree of Overlap (%)		
Study	Period	Radius	RR	P-value	Radius	RR	P-value	Radius	RR	P-value	PCR vs Microscopy	PCR vs RDT	RDT vs Microscopy
Junju	Overall	1.75	1.85	<0.001	1.75	2.69	<0.001	0.81	1.91	<0.001	100	45.9	45.9
	Overall*	1.07	2.23	<0.001	1.07	2.87	<0.001	1.07	2.61	<0.001	100	100	100
	2007	0.9	2.17	0.003	2.11	4.57	<0.001	2.38	4.99	<0.001	42.02	39.32	82.22
	2008	1.67	2.2	<0.001	1.76	2.22	0.002	1.58	3.92	<0.001	71.54	72.5	85.71
	2009	2.38	2.34	<0.001	1.54	3.4	<0.001	1.71	7.88	<0.001	68.79	55.84	73.64
	2010	1.77	2.01	<0.001	1.78	2.65	<0.001	1.97	3.76	<0.001	79.2	73.13	63.19
	2011	1.44	2.71	<0.001	1.72	6.28	<0.001	0.64	5.5	0.009	76.42	20.18	23.68
	2012	2.08	2.02	<0.001	1.29	2.7	<0.001	1.78	2.08	0.001	57.63	61.81	57.76
	2013	0.36	3.31	0.002	0.19	9.07	0.133	1.98	2.69	0.001	8.33	8.53	2.33
	2014	0	3.37	0.089	0.94	4.11	0.096	0.19	2.11	0.018	0	0	0
	2015	0.74	2.25	<0.001	0.63	3.31	<0.001	0.52	2.44	<0.001	25.42	48.65	22.64
	2015*	0.33	2.68	0.02	0.14	3.56	0.015	0.33	2.49	0.025	25.42	100	0
	2016	0	3.74	0.208	0.64	3.19	0.122	0.02	3.61	0.017	0	0	0
Ganze	Overall	12.12	4.14	<0.001	0.76	31	0.003	10.08	67.4	<0.001	2.75	75.61	2.8
Ngerenya	Overall	1.04	5.35	0.005	0	36.6	0.122	0	33.8	0.017	50	50	100
	2007-2010	0	8.96	0.213	1.65	8.11	0.758	0	60.69	0.059	0	100	0
	2010-2014	0.56	5.2	0.016	-	-	-	0.83	14.28	0.34	-	25	-

* includes significant secondary clusters

5.4.2 Association between parasite prevalence by PCR, microscopy and RDT

In all sites, and across all three grid sizes examined, there was a strong positive correlation between prevalence of parasitaemia measured by PCR and microscopy or RDT (i.e. geographical areas experiencing high malaria prevalence as measured by PCR were also more likely to be high when measured by microscopy or RDT). However, the associations were weaker in low transmission settings (Table 5.3 and Table 5.4).

Table 5.3: ***Association between parasite prevalence by PCR and microscopy at various grid sizes.***

Site	Year	Parasite Prevalence		0.5x0.5 km^2 Grid		1x1 km^2 Grid		2x2 km^2 Grid	
		PCR (%)	Microscopy (%)	Correlation (CI)	P-value	Correlation (CI)	P-value	Correlation (CI)	P-value
Junju Cohort	Overall	30.10	16.54	0.73 (0.70 - 0.76)	<0.001	0.81 (0.77 - 0.84)	<0.001	0.86 (0.82-0.89)	<0.001
	2007	29.82	16.27	0.70 (0.50 - 0.83)	<0.001	0.70 (0.37 - 0.88)	<0.001	0.83 (0.38-0.96)	0.005
	2008	47.51	29.33	0.79 (0.63 - 0.89)	<0.001	0.83 (0.62 - 0.94)	<0.001	0.93 (0.71-0.99)	<0.001
	2009	31.45	21.36	0.58 (0.32 - 0.76)	<0.001	0.82(0.58 - 0.93)	<0.001	0.90 (0.58-0.98)	<0.001
	2010	39.32	21.98	0.78 (0.71 - 0.84)	<0.001	0.76 (0.63 - 0.85)	<0.001	0.83 (0.65-0.92)	<0.001
	2011	26.93	15.48	0.69 (0.58 - 0.77)	<0.001	0.80 (0.69 - 0.87)	<0.001	0.88 (0.75-0.95)	<0.001
	2012	27.68	15.40	0.72 (0.62 - 0.79)	<0.001	0.79 (0.67 - 0.87)	<0.001	0.80 (0.59-0.91)	<0.001
	2013	19.42	7.89	0.69 (0.59 - 0.77)	<0.001	0.79 (0.67 -0.87)	<0.001	0.85 (0.68-0.93)	<0.001
	2014	30.32	14.76	0.73 (0.64 - 0.81)	<0.001	0.83 (0.73 - 0.89)	<0.001	0.91 (0.81-0.96)	<0.001
	2015	30.75	17.65	0.77 (0.61- 0.88)	<0.001	0.76 (0.49 - 0.90)	<0.001	0.81 (0.37-0.95)	0.005
	2016	23.51	11.26	0.46 (0.17 - 0.69)	0.004	0.48 (0.04 - 0.77)	0.036	0.47 (-0.22-0.85)	0.167
Ngerenya Cohort	Overall	2.04	0.21	0.37 (0.27-0.46)	<0.001	0.38 (0.26-0.48)	<0.001	0.40 (0.22-0.56	<0.001
Ganze Cohort	Overall	5.85	1.03	0.45 (0.34 - 0.55)	<0.001	0.45 (0.30 - 0.58)	<0.001	0.48 (0.28 - 0.63)	<0.001
	2012	7.73	1.81	0.51 (0.37 - 0.63)	<0.001	0.53 (0.35 - 0.68)	<0.001	0.60 (0.34 - 0.77)	<0.001
	2013	4.11	0.30	0.35 (0.17 - 0.51)	<0.001	0.30 (0.05 - 0.52)	0.0195	0.23 (-0.11 - 0.53)	0.1852

Table 5.4: ***Association between parasite prevalence by PCR and parasite prevalence by RDT at various grid sizes***

Site	Year	Parasite Prevalence		0.5x0.5 km^2 Grid		1x1 km^2 Grid		2x2 km^2 Grid	
		PCR (%)	RDT (%)	Correlation (CI)	P-value	Correlation (CI)	P-value	Correlation (CI)	P-value
Junju	Overall	30.1	20.2	0.58 (0.53 - 0.63)	<0.001	0.66 (0.60 - 0.71)	<0.001	0.67 (0.60-0.76)	<0.001
	2007	29.82	15.7	0.68 (0.46 - 0.82)	<0.001	0.69 (0.35 - 0.87)	<0.001	0.89 (0.54-0.98)	0.002
	2008	47.51	21.5	0.65 (0.42 - 0.81)	<0.001	0.53 (0.09 - 0.79)	0.021	0.93 (0.69-0.99)	<0.001
	2009	31.45	15.8	0.56 (0.30 - 0.75)	<0.001	0.69 (0.34 - 0.87)	0.001	0.87 (0.49-0.97)	0.002
	2010	39.32	13.9	0.52 (0.36 - 0.64)	<0.001	0.49 (0.25 - 0.67)	<0.001	0.35 (-0.08-0.66)	0.1045
	2011	26.93	5.3	0.56 (0.43 - 0.67)	<0.001	0.73 (0.59 - 0.83)	<0.001	0.77 (0.55-0.89)	<0.001
	2012	27.68	22.4	0.58 (0.45 - 0.69)	<0.001	0.66 (0.50 - 0.78)	<0.001	0.79 (0.57-0.90)	<0.001
	2013	19.42	14.6	0.63 (0.51 - 0.73)	<0.001	0.79 (0.67 - 0.87)	<0.001	0.89 (0.77-0.95)	<0.001
	2014	30.32	37.1	0.71 (0.61 - 0.79)	<0.001	0.88 (0.81 - 0.93)	<0.001	0.89 (0.77-0.95)	<0.001
	2015	30.75	32.8	0.55 (0.29 - 0.74)	<0.001	0.53 (0.13 - 0.78)	0.0135	0.86 (0.49-0.97)	0.002
	2016	23.51	28.9	0.54 (0.26 - 0.73)	<0.001	0.62 (0.23 - 0.84)	0.005	0.91 (0.66 - 0.98)	<0.001
Ganze	Overall	5.98	1.67	0.44 (0.33 - 0.54)	<0.001	0.47 (0.32 - 0.59)	<0.001	0.52 (0.33 - 0.67)	<0.001
	2012	8.18	2.99	0.56 (0.42 - 0.67)	<0.001	0.48 (0.28 - 0.64)	<0.001	0.59 (0.34 - 0.77)	<0.001
	2013	3.94	0.44	0.23 (0.04 - 0.41)	0.0170	0.44 (0.21 - 0.62)	<0.001	0.37 (0.03 - 0.63)	0.0327
Ngerenya	Overall	2.04	0.38	0.40 (0.22 - 0.56)	<0.001	0.85 (0.78 - 0.90)	<0.001	0.37 (0.18 - 0.53)	<0.001

5.4.3 Temporal stability of malaria transmission in the study sites

In the Junju site, the prevalences of parasitaemia within grids were predictive of the prevalence in the following year. The stability appeared to be greater for PCR and microscopy, which remained significant for intervals below 5 years, and less stable for RDT prevalences, which were significantly predictive of the prevalence in the following year and only up to 2 year intervals in this site.

In contrast, the prevalences of parasitaemia within grids in Ganze were not predictive for the following year by any measure (Table 5.5, Table 5.6 and Table 5.7) and such an analysis was not possible in Ngerenya due to low power resulting from limited number of malaria cases in the area. The findings on temporal stability were consistent across the 3 spatial scales used (0.5x0.5 km^2, 1x1 km^2 and 2x2 km^2).

Table 5.5*: Association between distribution of malaria parasite prevalence detected by microscopy, PCR and RDT within 0.5x0.5 km2 grid size over time intervals.*

		0.5x0.5 km^2 Grid					
Study Site	Interval between cluster (year)	Microscopy Analysis		PCR Analysis		RDT Analysis	
		Correlation (95%CI)	P-value	Correlation (95%CI)	P-value	Correlation (95%CI)	P-value
Junju	1	0.46 (0.40 - 0.52)	<0.001	0.40 (0.33-0.46)	<0.001	0.32 (0.25-0.39)	<0.001
	2	0.46 (0.40 - 0.53)	<0.001	0.34 (0.27-0.42)	<0.001	0.34 (0.26-0.41)	<0.001
	3	0.31 (0.22 - 0.39)	<0.001	0.28 (0.19-0.37)	<0.001	0.11 (0.02-0.21)	0.0233
	4	0.30 (0.20 - 0.40)	<0.001	0.30 (0.19-0.40)	<0.001	0.07 (-0.05-0.18)	0.2465
	5	0.31 (0.17 - 0.43)	<0.001	0.19 (0.05-0.33)	0.009	0.05 (-0.09-0.19)	0.4637
	6	0.25 (0.09 - 0.40)	0.0022	0.18 (0.02-0.34)	0.0313	0.11 (-0.06-0.27)	0.2092
	7	0.19 (0.001 - 0.37)	0.0494	0.17 (-0.02-0.35)	0.0748	0.18 (-0.02-0.35)	0.0719
	8	0.28 (0.05 - 0.49)	0.0207	0.11 (-0.13-0.34)	0.3554	0.16 (-0.09-0.38)	0.2038
	9	0.27 (-0.08 - 0.57)	0.122	-0.04 (-0.38 - 0.30)	0.8105	-0.04 (-0.30 - 0.38)	0.8253
Ganze	1	-0.05 (-0.40-0.31)	0.798	0.39 (0.04-0.65)	0.0308	-	-

Table 5.6*: Association between distribution of malaria parasite prevalence detected by microscopy, PCR and RDT within 1x1 km2 grid size over time intervals.*

		1x1 km^2 Grid					
Study Site	Interval between cluster (year)	Microscopy Analysis		PCR Analysis		RDT Analysis	
		Correlation (95%CI)	P-value	Correlation (95%CI)	P-value	Correlation (95%CI)	P-value
Junju	1	0.46 (0.37 - 0.54)	<0.001	0.41 (0.32-0.49)	<0.001	0.44 (0.35-0.52)	<0.001
	2	0.49 (0.40 - 0.58)	<0.001	0.40 (0.29-0.49)	<0.001	0.44 (0.34-0.53)	<0.001
	3	0.33 (0.21 - 0.45)	<0.001	0.34 (0.22-0.45)	<0.001	0.16 (0.03-0.29)	0.0175
	4	0.35 (0.21 - 0.48)	<0.001	0.38 (0.23-0.50)	<0.001	0.12 (-0.04-0.28)	0.1394
	5	0.27 (0.08 - 0.45)	0.006	0.15 (-0.05-0.34)	0.1403	0.03 (-0.17-0.22)	0.794
	6	0.29 (0.07 - 0.48)	0.01	0.19 (-0.03-0.39)	0.094	0.08 (-0.14-0.30)	0.4575
	7	0.20 (-0.05- 0.44)	0.1142	0.19 (-0.07-0.42)	0.1525	0.05 (-0.21-0.30)	0.7078
	8	0.23 (-0.09- 0.51)	0.1616	0.07 (-0.25-0.38)	0.6712	0.03 (-0.29-0.34)	0.871
	9	0.34 (-0.14- 0.69)	0.1574	0.04 (-0.42-0.48)	0.8789	-0.20 (-0.60-0.28)	0.4059
Ganze	1	-0.05 (-0.40-0.31)	0.798	0.39 (0.04-0.65)	0.0308	-	-

Table 5.7: ***Association between distribution of malaria parasite prevalence detected by microscopy, PCR and RDT within 2x2 km2 grid size over time intervals.***

Study Site	Interval between cluster (year)	2x2 km^2 Grid					
		Microscopy Analysis		PCR Analysis		RDT Analysis	
		Correlation (95%CI)	P-value	Correlation (95%CI)	P-value	Correlation (95%CI)	P-value
Junju cohort	1	0.46 (0.32-0.58)	<0.001	0.41 (0.26-0.53)	<0.001	0.43 (0.29-0.56)	<0.001
	2	0.55 (0.41-0.66)	<0.001	0.44 (0.29-0.58)	<0.001	0.50 (0.35-0.62)	<0.001
	3	0.44 (0.26-0.59)	<0.001	0.34 (0.15-0.51)	<0.001	0.18 (-0.02-0.37)	0.0843
	4	0.46 (0.25-0.63)	<0.001	0.48 (0.28-0.64)	<0.001	0.08 (-0.16-0.31)	0.532
	5	0.53 (0.29-0.71)	<0.001	0.34 (0.06-0.57)	0.0188	0.11 (-0.19-0.38)	0.4836
	6	0.47 (0.18-0.69)	0.003	0.32 (-0.01-0.59)	0.0505	0.27 (-0.07-0.55)	0.1179
	7	0.48 (0.12-0.73)	0.0111	0.65 (0.35-0.82)	<0.001	0.22 (-0.17-0.56)	0.2651
	8	0.33 (-0.16-0.69)	0.1788	0.27 (-0.22-0.66)	0.2732	0.34 (-0.15-0.70)	0.1659
	9	0.54 (-0.19-0.89)	0.1318	0.74 (0.14-0.94)	0.024	0.34 (-0.42-0.82)	0.3721
Ganze cohort	1	0.35 (-0.08- 0.67)	0.1075	0.30 (-0.14-0.64)	0.1712	-	-

5.5 Discussion

P. falciparum parasite prevalence has frequently been used as a marker of transmission intensity and is widely used in detection of hotspots of asymptomatic parasitaemia. However, the estimated prevalence of parasitaemia has been shown to vary substantially with the diagnostic tool used. PCR and other molecular techniques are significantly more sensitive than microscopy and RDT for detection of malaria parasites, especially at lower transmission intensities where parasite densities are lower *(Okell et al. 2009, Okell et al. 2012)*. This study examines the micro-epidemiology of malaria transmission in three sites on the Kenyan coast experiencing varying transmission intensities.

There was substantial heterogeneity of malaria transmission in the three sites, as has been previously described *(Bejon et al. 2010)*. Hotspots were detected by PCR, RDT and microscopy, and were statistically significant for all sites except by microscopy in the Ngerenya site. When all years from the Junju site were pooled for spatial analysis, hotspots by PCR completely overlapped with hotspots by microscopy and partially overlapped with RDT. However, an analysis of individual year by year data showed some variation in the degree of overlap (Table 5.1). Overlap became less marked in later years, coinciding with reductions in transmission intensity *(O'Meara et al. 2008)*, and little overlap was noted in Ganze, where transmission is lower *(Kangoye et al. 2016)*. It is unsurprising that hotspots of the different malaria species overlapped geographically since they are transmitted by similar vectors.

There were significant correlations between PCR and microscopy, and between PCR and RDT parasite prevalences within grid cells imposed on the data at three different spatial scales. The correlations were stronger in Junju than in Ngerenya and Ganze (Table 5.2). The prevalence of infection was below 2% in Ngerenya and Ganze. Taking the findings on degree of overlap of

hotspots in the different transmission settings and the correlation between parasite prevalence together, I conclude that hotspots detected by PCR are likely to occur in the same geographical areas as those detected by microscopy at moderate transmission intensities. However, the degree to which they overlap is lessened when transmission is less intense.

As would be expected, PCR densities were lower than microscopy densities *(Bejon et al. 2006)*, and the average densities by PCR were lower in low transmission settings (Ngerenya and Ganze) compared to the moderate transmission setting (Junju). Moreover, the proportion of PCR positive cases that were positive by microscopy were highest in Junju, followed by Ganze and Ngerenya in that order (Table 5.1). My findings suggest that microscopy and RDT misses a larger proportion of infections in low transmission areas (Ngerenya and Ganze) compared to the one moderate transmission setting in Junju, and may explain why PCR becomes more important in detecting hotspots at lower transmission intensities.

I observed stable hotspots of asymptomatic parasitaemia in the Junju cohort but not in Ganze, and I was not sufficiently powered to assess stability of hotspots in Ngerenya. Hotspots were similarly stable when detected by PCR or microscopy but not RDT (Table 5.3). The advent of HRP2 dependent RDTs greatly expanded access to malaria diagnostics tools because of low cost and ease of applicability in the field, but the sensitivity of this technique is lower than that of PCR and may be comparable to the sensitivity of routine microscopy *(WHO 2012b)*. In addition, HRP2 antigen can circulate in blood for weeks after treatment leading to false positives, and recent studies show that some *P. falciparum* parasites do not express the HRP2 protein leading to false negatives *(Cheng et al. 2014)*. These factors potentially result in poorer discrimination for the location of hotspots, explaining the lack of long-term stability of hotspots detected by RDT. Furthermore, hotspots defined by RDT did not consistently overlap the PCR

or microscopy hotspots. I conclude that although RDTs have a firmly established place in diagnosis of acute fever and malaria indicator surveys *(Murray et al. 2008, WHO 2012b)*, their utility for fine-scale mapping of hotspots is less clear.

The main limitation of this study is that data were collected from geographical areas of close proximity on the Kenyan coast. However, these geographical areas captured a range of transmission intensities and during a period when transmission was falling *(Mogeni et al. 2016)*. Although the Ngerenya dataset (i.e. a site with low transmission intensity) was large (n=2286), there were few positive cases (Table 5.1) and hence limited power to describe and compare hotspots. Microscopy has low sensitivity in detecting minority species in mixed infections. In this study only *P. falciparum* specific PCR was used and this could have led to non-*falciparum* infections being missed and therefore underestimated the prevalence of the minority species and the position, size and significance of the hotspots.

Clinical malaria case monitoring has also been used to identify hotspots of malaria transmission *(Kangoye et al. 2016)*. However, this may be less sensitive in identifying stable hotspots of malaria where substantial immunity in the population offsets the risk of clinical malaria *(Bejon et al. 2010)* and even at low transmission intensity, hotspots determined by PCR do not overlap with microscopy hotspots *(Kangoye et al. 2016)*. Hence, PCR monitoring of asymptomatic infection may identify hotspots that would not be detected by monitoring clinical cases and may be useful for pre-elimination surveillance.

5.6 Implications of the findings

Malaria control programs increasingly need to adopt targeted malaria control at low transmission intensities. My findings suggest that PCR, RDT and microscopy can potentially

determine hotspots at moderate transmission intensities, but PCR testing has a diagnostic advantage as transmission intensity falls. Therefore, Malaria control programs should consider PCR testing when the prevalence of infection is low.

Chapter Six

6 Concluding remarks and recommendations

6.1 Summary of findings

Parts of sub-Saharan Africa have witnessed declines in malaria transmission intensity over the past two decades. Spatial heterogeneity is expected to be more marked as transmission intensity declines and therefore presents an opportunity for targeted control. However, this should be accompanied by an understanding of the factors predicting malaria heterogeneity. Furthermore, it is predicted that declining transmission intensity may result to a shift in age of susceptibility to malaria with important consequences in case fatality rates. The analyses presented in chapter 3 of this thesis examines some of these features using a detailed 25-year longitudinal hospital surveillance dataset with the aim of describing the spatial-temporal patterns of malaria transmission in Kilifi County. I conducted further analysis of trends of malaria positive fraction by age group and the effect of environmental factors, personal and community ITN use, and age on malaria heterogeneity. The key findings were: -

- Malaria transmission intensity in Kilifi County is spatially and temporally heterogeneous. Hospital presentation with malaria parasites declined from around the year 2000 to 2009 and increased through to 2014. The decline in transmission intensity occurred before the mass distribution of ITN and the practical implementation of the government policy change over from chloroquine to ACT as the first line mode of treatment against uncomplicated malaria. Geographical heterogeneity was greater after the decline keeping with previous observations. Furthermore, the stability of malaria transmission at the locational level declined with declining transmission intensity such

that the negative association between MPF and age of susceptibility at the post decline period was non-significant.

- During the high transmission period, young children predominantly presented to hospital for admission with malaria parasites in their blood. However, as transmission intensity declined, largely older children presented with malaria for admission. Fortunately, there was no clear trend towards increasing mortality as would be predicted. This result may not be definitive given several unmeasured potential confounders that likely mask the true trend in mortality over time. For instance, a) increasing hospital accessibility over time leading to more timely presentation for treatment and b) improved malaria case management over time because of implemented research findings.
- The prevalence of ITN use among the whole population within a 2-kilometre radius of the admitted child's residence was strongly predictive of protection. High community ITN coverages were associated with lower malaria cases.
- The enhanced vegetation index (EVI) was predictive of malaria but explained a small fraction of the variability in the outcome variable. Overall, the variability explained by the model was ~23%.

Geographically defined clusters (hotspots) are a consistent feature of malaria transmission in sub-Saharan Africa. The utility of hotspots to malaria control programmes is at moderate to low transmission intensity where hotspots are more marked and predicted to complicate elimination efforts. Although hotspots have been detected in most epidemiological studies, the potential of targeting them through cluster randomised controlled studies has not been assessed comprehensively to determine their utility in the field. This is partly because rational design of cluster randomised control interventions need detailed descriptions of the properties of hotspots

and the methods used to detect them. These properties of hotspots and the effects of diagnostic tools on hotspots are not well understood and compared over a variety of transmission settings to assess their reproducibility. The analyses presented in chapter 4 and chapter 5 of this thesis addressed some of these issues.

Key findings: -

- The risk ratio of malaria within the hotspots was higher at low MPF suggesting that spatial heterogeneity becomes gradually marked at low transmission intensities, keeping with results from chapter 3. However, the strength of evidence (p-values) increased from low MPFs to moderate MPFs and then declined towards high MPFs further suggesting that although spatial heterogeneity becomes more apparent at low transmission intensities, the power to detect hotspots during low transmission becomes low as cases become more sparse.
- Previous analysis of hotspots in Kilifi County on the Kenyan coast identified two types of hotspots; a) stable hotspots of asymptomatic malaria and b) unstable hotspots of febrile malaria. I examined the stability of hotspots using 4 longitudinal studies conducted outside Kilifi county to assess generalizability. Both hotspots of asymptomatic parasitaemia and hotspots of febrile malaria were unstable suggesting that stability of hotspots of asymptomatic malaria is not a reproducible feature in all transmission settings.
- Micro-variation of malaria transmission was negatively associated with age of susceptibility in most datasets, suggesting that, during high transmission intensity, children acquire immunity rapidly due to intense exposure when they are younger and hence do not present with symptomatic malaria when they are older and vice versa. I

assessed the association between micro-variation of symptomatic malaria with age of susceptibility for each dataset and combined the estimates in a meta-analysis at three spatial scales determined *a priori*. Heterogeneity between studies (I^2) declined with increasing spatial scale while the pooled correlation estimate became larger at relatively coarse spatial scales keeping with previous studies *(Bejon et al. 2014)*. In addition, there were significant positive spatial autocorrelations in age of susceptibility to febrile malaria in most datasets of febrile malaria, thus providing further evidence that micro-variation of transmission intensity may have led to variation in the degree of acquisition of clinical immunity.

The prevalence of asymptomatic parasitaemia varies considerably between diagnostic tools *(Okell et al. 2009, Okell et al. 2012)*. The aim of the analysis presented in chapter 5 *(Mogeni et al. 2017b)* was to assess the degree with which hotspots detected by RDT, PCR and microscopy analysis overlap geographically.

Key findings: -

- Substantial heterogeneity of malaria transmission was observed in the three sites, as previously reported *(Bejon et al. 2010)*. *P. falciparum* was detected in the three sites examined (Junju, Ngerenya and Ganze cohorts), however, *P. ovale* and *P. malariae* were only detected in the Junju site. Hotspots of *P. falciparum* examined by microscopy overlapped with hotspots of *P. ovale* and *P. malariae* but there was no reported case of *P. vivax* in any of the three sites examined.
- The degree of overlap between diagnostic tools decreased with decreasing transmission intensity. Hotspots detected by PCR and microscopy remained stable for over five years, however, hotspots detected by RDTs were significantly stable for up to 2 years.

The studies presented in this thesis provides important empirical evidence on the likely outcome following declined malaria transmission intensity and the importance of universal coverage with ITNs. This information emphasizes the need for continued investment on malaria control interventions even when transmission intensity is very low. The studies contribute to the limited empirical evidence of increased heterogeneity as transmission intensity decline. Furthermore, important recommendations for the malaria control programs are deduced; 1) PCR testing is needed for detection of asymptomatic malaria during low transmission intensities 2) Targeting of hotspots of malaria transmission may achieve greater impact when transmission intensity is low (i.e. when the PfPR is below 10%).

Alternative definitions of hotspots

A malaria hotspot can also be defined as an area where the malaria risk is highly likely to exceed a threshold considered to be relevant to policy. A key challenge with policy thresholds is in the determination of a universally acceptable cutoff that cuts across the spectrum of malaria transmission intensities. The definition could alternatively be based on prior theoretical assumptions. For instance, a threshold that includes 20% of the analysed population in the hotspot *(Stresman et al. 2017)* is based on the theoretical 80–20 assumption where 20% of the population has been shown to experience 80% of the disease episodes *(Woolhouse et al. 1997a)*. Although this threshold may be applicable in some settings, site specific variations in distributions of infection may exist and the most appropriate proportion of the population to include in a hotspot may vary *(Stresman et al. 2017)*.

A hotspot of malaria transmission can also be defined as an area where the difference between the local sum of positive malaria cases for a given location and its neighbours is larger than the expected as calculated using the Getis Ord G_i* statistic. The Getis Ord Gi* statistic yields

standardised Z-scores and is defined as the ratios of the local sum of the values in the vicinity of a given distance to the sum of all values. When the local sum is different than the expected local sum, and the difference is too large to be the result of random chance, a statistically significant Z-score results. The positive Z-scores indicate high cases of malaria (hotspots) while negative Z-scores indicate low malaria cases (cold spots) *(Getis and Ord 1992)*. The significance of a hotspot can be obtained from an optimized hotspot analysis function in ArcGIS (Ver.10.2, ESRI Inc., CA, USA) using threshold Z-scores *(Izumi et al. 2015)*. For instance, Gwitira et al used a Z-score ≥1.96 as a cut-off to define a hotspot based on the critical Z-score values from 95% confidence level *(Gwitira et al. 2018)*. Although Getis-Ord Gi* statistic has been used in literature to detect hotspots, it suffers from the problem of multiple testing *(Gwitira et al. 2018)*, a longstanding difficulty in determining hotspots in the absence of non-arbitrary geographical "bins" for data, as recognized by Openshaw *(Openshaw et al. 1987)*.

6.2 Recommendations for future studies

Although there was no clear trend towards increasing mortality in Kilifi following initial reductions in transmission intensity (chapter 3), parallel factors may contribute to the observed outcome. For instance, increased accessibility or hospital acceptance and improved case management may mask the true trend in mortality over time, if all factors were kept constant. Further analysis of malaria phenotypes over time may be required to give insight on the impact of the age-shifting burden of disease to older children on the severity of malaria.

The rate of ITNs loss within the KHDSS area following the mass distribution of nets is a cause of concern. This work can be extended to assess reasons for ITN loss/ non ITN use in the community through focused group discussions and community engagement to improve ITN uptake.

Further analysis on the stability of hotspots during the dry and rainy seasons will likely provide useful information on the contribution of seasonality. Previous analysis shows that hotspots are maintained during dry seasons and rainy seasons *(Bejon et al. 2010, Bousema et al. 2012b)*, however, there is little empirical evidence on the degree with which such hotspots overlap. The analysis of year to year variations presented in chapter 4 and 5 masks the potential role of seasonality. A more temporally fine-grained analysis to examine seasonal signals would have been a useful addition to this work, however, sample size constraints made it difficult to carry out monthly analysis for the limited longitudinal datasets that were available.

Hotspots of malaria transmission could be predicted using remote sensing data extracted from NASA satellite (aqua and terra) *(Bejon et al. 2010)*. Data from these satellites senses various environmental signals reflecting vegetation density, water/moisture and temperature. I identified that EVI (enhanced vegetation index) weakly predicted malaria cases in Kilifi County in Chapter 3. A further extension to chapter 4 would entail an analysis of the correlation between the satellite data and malaria prevalence or MPF using regression models. The estimates can then be pooled to obtain overall effect sizes and a measure of heterogeneity between studies. A further assessment of trend in study specific effect size (for the various environmental covariates) over transmission intensity will be a valuable addition to the current literature assessing the effect environmental covariates at varying transmission intensities.

Significant hotspots of malaria transmission can be detected within larger hotspots up to the homestead level *(Bejon et al. 2014)*. This suggest that, the size of a hotspot will largely depend on the size of the area that is being examined. These feature of hotspots makes it difficult to determine the size of a hotspot that could be targeted if the whole foci of transmission were to

be studied. I recommend genomic studies to assess the degree of parasite mixing in space and time to determine the distances beyond which parasite mixing is unlikely.

An important extension to chapter 5 will entail an analysis of the correlation between overlap of hotspots by diagnostic tools and temporal stability. This would require large assembled longitudinal datasets from which parameters describing both the degree of overlap and stability of hotspots could be computed and the correlations between these parameters assessed.

6.3 Recommendations for malaria control programmes

In conclusion, I recommend continued monitoring of malaria cases and related outcomes following declining malaria transmission intensity. Malaria control programmes should continue investing in malaria control interventions even when malaria transmission intensity appears to be low to prevent a likely "rebound" in malaria among older children as a population emerges with less immunity than was previously the case. ITN use remains an effective malaria control tool and achieving universal coverage should be prioritized. Key groups to target to achieve universal coverage are older children and adolescents.

Heterogeneity of malaria transmission becomes more apparent as transmission intensity declines and is particularly marked at transmission intensities <10%. I propose that malaria transmission hotspots should be targeted when the overall parasite prevalence is low (<~10%), as my data suggest slight more modest clustering of malaria cases in hotspots at high transmission intensity. Many sub-Saharan Africa countries experience transmission intensities considerably above this threshold and may not benefit from hotspots targeted interventions. However, some countries in sub-Saharan Africa are already in the elimination stage and may benefit from targeted interventions. Mathematical modelling studies could guide on the most cost effective combination of interventions and coverage levels to achieve elimination. The

stability of hotspots may not be a reproducible property of malaria transmission hotspots in sub-Saharan Africa and should therefore be assessed prior to rollout of interventions, and surveillance should therefore be continuously done to guide malaria control. Given the evidence on hotspots properties discussed, there is a need to design field trials to assess the impact of hotspot targeted interventions and the cost implication in areas experiencing low transmission intensities. Finally, I recommend Polymerase Chain Reaction for the identification and targeting of asymptomatic hotspots of malaria transmission when cross-sectional surveys are used in areas of very low transmission intensity, since RDT or microscopy seem to identify very similar hotspots at low to moderate transmission intensity.

At the pre-elimination *(Cohen et al. 2010)* stage of malaria control, hotspots of malaria transmission are likely to be less stable and to lead to mainly symptomatic malaria cases due to reduced immunity among the population resulting from reduced exposure. At this stage malaria control programmes may consider using health-care based surveillance systems for targeting intervention. For instance, a symptomatic case presenting to hospital may be used to map out areas requiring targeted mass drug administration (MDA) in which case close contacts, households or villages of the index case are targeted *(Hsiang et al. 2013)*. Alternatively, whole population MDA could be considered to clear residual transmission in areas of very low transmission intensity *(von Seidlein and Greenwood 2003, Cotter et al. 2013)*. However, this approach will likely require an organized effort by neighboring countries and would be more feasible at a regional level as opposed to individual countries due to cross border human mobility and border vector dispersal. At low transmission intensity, MDA will likely result in treatment of many uninfected individuals, and this could be avoided by using a screening test to target treatment (i.e. mass screen and treat or MSAT). Countrywide MSAT will be hampered by the

apparent detection limitations of the available diagnostic tools and the amount of resources set aside for malaria interventions.

7 References

Afrane, Y. A., A. K. Githeko and G. Yan (2012). "The ecology of Anopheles mosquitoes under climate change: case studies from the effects of deforestation in East African highlands." Ann N Y Acad Sci **1249**: 204-210.

Allison, P. D. (2000). "Multiple imputation for missing data: A cautionary tale." Sociological methods & research **28**(3): 301-309.

Alonso, P. L., S. W. Lindsay, J. R. Armstrong, M. Conteh, A. G. Hill, P. H. David, G. Fegan, A. de Francisco, A. J. Hall, F. C. Shenton and et al. (1991). "The effect of insecticide-treated bed nets on mortality of Gambian children." Lancet **337**(8756): 1499-1502.

Alonso, P. L., S. W. Lindsay, J. R. Armstrong Schellenberg, K. Keita, P. Gomez, F. C. Shenton, A. G. Hill, P. H. David, G. Fegan, K. Cham and et al. (1993). "A malaria control trial using insecticide-treated bed nets and targeted chemoprophylaxis in a rural area of The Gambia, west Africa. 6. The impact of the interventions on mortality and morbidity from malaria." Trans R Soc Trop Med Hyg **87 Suppl 2**: 37-44.

Altman, D. G. and M. Bland (1997). "Statistics notes: units of analysis." Bmj **314**(7098): 1874.

Amexo, M., R. Tolhurst, G. Barnish and I. Bates (2004). "Malaria misdiagnosis: effects on the poor and vulnerable." Lancet **364**(9448): 1896-1898.

Anselin, L. (1995). "Local indicators of spatial association—LISA." Geographical analysis **27**(2): 93-115.

Aponte, J. J., D. Schellenberg, A. Egan, A. Breckenridge, I. Carneiro, J. Critchley, I. Danquah, A. Dodoo, R. Kobbe and B. Lell (2009). "Efficacy and safety of intermittent preventive treatment with sulfadoxine-pyrimethamine for malaria in African infants: a pooled analysis of six randomised, placebo-controlled trials." The Lancet **374**(9700): 1533-1542.

Ashley, E. A., M. Dhorda, R. M. Fairhurst, C. Amaratunga, P. Lim, S. Suon, S. Sreng, J. M. Anderson, S. Mao, B. Sam, C. Sopha, C. M. Chuor, C. Nguon, S. Sovannaroth, S. Pukrittayakamee, P. Jittamala, K. Chotivanich, K. Chutasmit, C. Suchatsoonthorn, R. Runcharoen, T. T. Hien, N. T. Thuy-Nhien, N. V. Thanh, N. H. Phu, Y. Htut, K. T. Han, K. H. Aye, O. A. Mokuolu, R. R. Olaosebikan, O. O. Folaranmi, M. Mayxay, M. Khanthavong, B. Hongvanthong, P. N. Newton, M. A. Onyamboko, C. I. Fanello, A. K. Tshefu, N. Mishra, N. Valecha, A. P. Phyo, F. Nosten, P. Yi, R. Tripura, S. Borrmann, M. Bashraheil, J. Peshu, M. A. Faiz, A. Ghose, M. A. Hossain, R. Samad, M. R. Rahman, M. M. Hasan, A. Islam, O. Miotto, R. Amato, B. MacInnis, J. Stalker, D. P. Kwiatkowski, Z. Bozdech, A. Jeeyapant, P. Y. Cheah, T. Sakulthaew, J. Chalk, B. Intharabut, K. Silamut, S. J. Lee, B. Vihokhern, C. Kunasol, M. Imwong, J. Tarning, W. J. Taylor, S. Yeung, C. J. Woodrow, J. A. Flegg, D. Das, J. Smith, M. Venkatesan, C. V. Plowe, K. Stepniewska, P. J. Guerin, A. M. Dondorp, N. P. Day and N. J. White (2014). "Spread of artemisinin resistance in Plasmodium falciparum malaria." N Engl J Med **371**(5): 411-423.

Ashley, E. A. and N. J. White (2014). "The duration of Plasmodium falciparum infections." Malar J **13**: 500.

Atroosh, W. M., H. M. Al-Mekhlafi, M. A. Mahdy, R. Saif-Ali, A. M. Al-Mekhlafi and J. Surin (2011). "Genetic diversity of Plasmodium falciparum isolates from Pahang, Malaysia based on MSP-1 and MSP-2 genes." Parasit Vectors **4**: 233.

Auchincloss, A. H., S. Y. Gebreab, C. Mair and A. V. Diez Roux (2012). "A review of spatial methods in epidemiology, 2000–2010." Annual review of public health **33**: 107-122.

Baidjoe, A. Y., J. Stevenson, P. Knight, W. Stone, G. Stresman, V. Osoti, E. Makori, C. Owaga, W. Odongo, P. China, S. Shagari, S. Kariuki, C. Drakeley, J. Cox and T. Bousema (2016). "Factors associated with high heterogeneity of malaria at fine spatial scale in the Western Kenyan highlands." Malar J **15**: 307.

Bannister-Tyrrell, M., K. Verdonck, S. Hausmann-Muela, C. Gryseels, J. Muela Ribera and K. Peeters Grietens (2017). "Defining micro-epidemiology for malaria elimination: systematic review and meta-analysis." Malar J **16**(1): 164.

Baragatti, M., F. Fournet, M. C. Henry, S. Assi, H. Ouedraogo, C. Rogier and G. Salem (2009). "Social and environmental malaria risk factors in urban areas of Ouagadougou, Burkina Faso." Malar J **8**: 13.

Bautista, C. T., A. S. Chan, J. R. Ryan, C. Calampa, M. H. Roper, A. W. Hightower and A. J. Magill (2006). "Epidemiology and spatial analysis of malaria in the Northern Peruvian Amazon." Am J Trop Med Hyg **75**(6): 1216-1222.

Bayoh, M. N. and S. W. Lindsay (2003). "Effect of temperature on the development of the aquatic stages of Anopheles gambiae sensu stricto (Diptera: Culicidae)." Bull Entomol Res **93**(5): 375-381.

Bayoh, M. N. and S. W. Lindsay (2004). "Temperature-related duration of aquatic stages of the Afrotropical malaria vector mosquito Anopheles gambiae in the laboratory." Med Vet Entomol **18**(2): 174-179.

Beard, J. (2006). "DDT and human health." Sci Total Environ **355**(1-3): 78-89.

Beck, H. P., I. Felger, W. Huber, S. Steiger, T. Smith, N. Weiss, P. Alonso and M. Tanner (1997). "Analysis of multiple Plasmodium falciparum infections in Tanzanian children during the phase III trial of the malaria vaccine SPf66." J Infect Dis **175**(4): 921-926.

Beier, J. C. (1998). "Malaria parasite development in mosquitoes." Annu Rev Entomol **43**: 519-543.

Bejon, P., L. Andrews, A. Hunt-Cooke, F. Sanderson, S. C. Gilbert and A. V. Hill (2006). "Thick blood film examination for Plasmodium falciparum malaria has reduced sensitivity and underestimates parasite density." Malar J **5**: 104.

Bejon, P., J. A. Berkley, T. Mwangi, E. Ogada, I. Mwangi, K. Maitland, T. Williams, J. A. Scott, M. English, B. S. Lowe, N. Peshu, C. R. Newton and K. Marsh (2007). "Defining childhood severe falciparum malaria for intervention studies." PLoS Med **4**(8): e251.

Bejon, P., T. N. Williams, A. Liljander, A. M. Noor, J. Wambua, E. Ogada, A. Olotu, F. H. Osier, S. I. Hay, A. Farnert and K. Marsh (2010). "Stable and unstable malaria hotspots in longitudinal cohort studies in Kenya." PLoS Med **7**(7): e1000304.

Bejon, P., T. N. Williams, C. Nyundo, S. I. Hay, D. Benz, P. W. Gething, M. Otiende, J. Peshu, M. Bashraheil, B. Greenhouse, T. Bousema, E. Bauni, K. Marsh, D. L. Smith and S. Borrmann (2014). "A micro-epidemiological analysis of febrile malaria in Coastal Kenya showing hotspots within hotspots." Elife **3**: e02130.

Ben, R., K., O. Tebourbi, R. Krichah and M. Sakly (2001). "Reproductive toxicity of DDT in adult male rats." Hum Exp Toxicol **20**(8): 393-397.

Bennett, A., D. Bisanzio, J. O. Yukich, B. Mappin, C. A. Fergus, M. Lynch, R. E. Cibulskis, S. Bhatt, D. J. Weiss, E. Cameron, P. W. Gething and T. P. Eisele (2017). "Population coverage of artemisinin-based combination treatment in children younger than 5 years with fever and Plasmodium falciparum infection in Africa, 2003-2015: a modelling study using data from national surveys." Lancet Glob Health **5**(4): e418-e427.

Besag, J. and J. Newell (1991). "The detection of clusters in rare diseases." Journal of the Royal Statistical Society. Series A (Statistics in Society): 143-155.

Bhatt, S., D. J. Weiss, E. Cameron, D. Bisanzio, B. Mappin, U. Dalrymple, K. E. Battle, C. L. Moyes, A. Henry, P. A. Eckhoff, E. A. Wenger, O. Briet, M. A. Penny, T. A. Smith, A. Bennett, J. Yukich, T. P. Eisele, J. T. Griffin, C. A. Fergus, M. Lynch, F. Lindgren, J. M. Cohen, C. L. Murray, D. L. Smith, S. I. Hay, R. E. Cibulskis and P. W. Gething (2015a). "The effect of malaria control on Plasmodium falciparum in Africa between 2000 and 2015." Nature **526**(7572): 207-211.

Bhatt, S., D. J. Weiss, B. Mappin, U. Dalrymple, E. Cameron, D. Bisanzio, D. L. Smith, C. L. Moyes, A. J. Tatem, M. Lynch, C. A. Fergus, J. Yukich, A. Bennett, T. P. Eisele, J. Kolaczinski, R. E. Cibulskis, S. I. Hay and P. W. Gething (2015b). "Coverage and system efficiencies of insecticide-treated nets in Africa from 2000 to 2017." Elife **4**.

Bhattarai, A., A. S. Ali, S. P. Kachur, A. Martensson, A. K. Abbas, R. Khatib, A. W. Al-Mafazy, M. Ramsan, G. Rotllant, J. F. Gerstenmaier, F. Molteni, S. Abdulla, S. M. Montgomery, A. Kaneko and A. Bjorkman (2007). "Impact of artemisinin-based combination therapy and insecticide-treated nets on malaria burden in Zanzibar." PLoS Med **4**(11): e309.

Bi, Y., W. Hu, H. Liu, Y. Xiao, Y. Guo, S. Chen, L. Zhao and S. Tong (2012). "Can slide positivity rates predict malaria transmission?" Malar J **11**: 117.

Binka, F. N., A. Kubaje, M. Adjuik, L. A. Williams, C. Lengeler, G. H. Maude, G. E. Armah, B. Kajihara, J. H. Adiamah and P. G. Smith (1996). "Impact of permethrin impregnated bednets on child mortality in Kassena-Nankana district, Ghana: a randomized controlled trial." Trop Med Int Health **1**(2): 147-154.

Birley, M. H. and J. D. Charlewood (1987). "Sporozoite rate and malaria prevalence." Parasitol Today **3**(8): 231-232.

Bousema, T., R. R. Dinglasan, I. Morlais, L. C. Gouagna, T. van Warmerdam, P. H. Awono-Ambene, S. Bonnet, M. Diallo, M. Coulibaly, T. Tchuinkam, B. Mulder, G. Targett, C. Drakeley, C. Sutherland, V. Robert, O. Doumbo, Y. Toure, P. M. Graves, W. Roeffen, R. Sauerwein, A. Birkett, E. Locke, M. Morin, Y. Wu and T. S. Churcher (2012a). "Mosquito feeding assays to determine the infectiousness of naturally infected Plasmodium falciparum gametocyte carriers." PLoS One **7**(8): e42821.

Bousema, T., C. Drakeley, S. Gesase, R. Hashim, S. Magesa, F. Mosha, S. Otieno, I. Carneiro, J. Cox, E. Msuya, I. Kleinschmidt, C. Maxwell, B. Greenwood, E. Riley, R. Sauerwein, D. Chandramohan and R. Gosling (2010a). "Identification of hot spots of malaria transmission for targeted malaria control." J Infect Dis **201**(11): 1764-1774.

Bousema, T., J. T. Griffin, R. W. Sauerwein, D. L. Smith, T. S. Churcher, W. Takken, A. Ghani, C. Drakeley and R. Gosling (2012b). "Hitting hotspots: spatial targeting of malaria for control and elimination." PLoS Med **9**(1): e1001165.

Bousema, T., L. Okell, I. Felger and C. Drakeley (2014). "Asymptomatic malaria infections: detectability, transmissibility and public health relevance." Nat Rev Microbiol **12**(12): 833-840.

Bousema, T., G. Stresman, A. Y. Baidjoe, J. Bradley, P. Knight, W. Stone, V. Osoti, E. Makori, C. Owaga, W. Odongo, P. China, S. Shagari, O. K. Doumbo, R. W. Sauerwein, S. Kariuki, C. Drakeley, J. Stevenson and J. Cox (2016). "The Impact of Hotspot-Targeted Interventions on Malaria Transmission in Rachuonyo South District in the Western Kenyan Highlands: A Cluster-Randomized Controlled Trial." PLoS Med **13**(4): e1001993.

Bousema, T., R. M. Youssef, J. Cook, J. Cox, V. A. Alegana, J. Amran, A. M. Noor, R. W. Snow and C. Drakeley (2010b). "Serologic markers for detecting malaria in areas of low endemicity, Somalia, 2008." Emerg Infect Dis **16**(3): 392-399.

Brabin, B. J. (1983). "An analysis of malaria in pregnancy in Africa." Bull World Health Organ **61**(6): 1005-1016.

Brooker, S., S. Clarke, J. K. Njagi, S. Polack, B. Mugo, B. Estambale, E. Muchiri, P. Magnussen and J. Cox (2004). "Spatial clustering of malaria and associated risk factors during an epidemic in a highland area of western Kenya." Trop Med Int Health **9**(7): 757-766.

Burkot, T. R., P. M. Graves, R. Paru, D. Battistutta, A. Barnes and A. Saul (1990). "Variations in malaria transmission rates are not related to anopheline survivorship per feeding cycle." Am J Trop Med Hyg **43**(4): 321-327.

Carter, R. and K. N. Mendis (2002). "Evolutionary and Historical Aspects of the Burden of Malaria." Clinical Microbiology Reviews **15**(4): 564-594.

Ceesay, S. J., K. A. Bojang, D. Nwakanma, D. J. Conway, O. A. Koita, S. O. Doumbia, D. Ndiaye, T. F. Coulibaly, M. Diakite, S. F. Traore, M. Coulibaly, J. L. Ndiaye, O. Sarr, O. Gaye, L. Konate,

N. Sy, B. Faye, O. Faye, N. Sogoba, M. Jawara, A. Dao, B. Poudiougou, S. Diawara, J. Okebe, L. Sangare, I. Abubakar, A. Sissako, A. Diarra, M. Keita, B. Kandeh, C. A. Long, R. M. Fairhurst, M. Duraisingh, R. Perry, M. A. Muskavitch, C. Valim, S. K. Volkman, D. F. Wirth and D. J. Krogstad (2012). "Sahel, savana, riverine and urban malaria in West Africa: Similar control policies with different outcomes." Acta Trop **121**(3): 166-174.

Ceesay, S. J., C. Casals-Pascual, J. Erskine, S. E. Anya, N. O. Duah, A. J. Fulford, S. S. Sesay, I. Abubakar, S. Dunyo, O. Sey, A. Palmer, M. Fofana, T. Corrah, K. A. Bojang, H. C. Whittle, B. M. Greenwood and D. J. Conway (2008). "Changes in malaria indices between 1999 and 2007 in The Gambia: a retrospective analysis." Lancet **372**(9649): 1545-1554.

Ceesay, S. J., C. Casals-Pascual, D. C. Nwakanma, M. Walther, N. Gomez-Escobar, A. J. Fulford, E. N. Takem, S. Nogaro, K. A. Bojang, T. Corrah, M. C. Jaye, M. A. Taal, A. A. Sonko and D. J. Conway (2010). "Continued decline of malaria in The Gambia with implications for elimination." PLoS One **5**(8): e12242.

Chandre, F., F. Darrier, L. Manga, M. Akogbeto, O. Faye, J. Mouchet and P. Guillet (1999). "Status of pyrethroid resistance in Anopheles gambiae sensu lato." Bull World Health Organ **77**(3): 230-234.

Chen, I., S. E. Clarke, R. Gosling, B. Hamainza, G. Killeen, A. Magill, W. O'Meara, R. N. Price and E. M. Riley (2016). ""Asymptomatic" Malaria: A Chronic and Debilitating Infection That Should Be Treated." PLoS Med **13**(1): e1001942.

Cheng, H., W. Yang, W. Kang and C. Liu (1995). "Large-scale spraying of bednets to control mosquito vectors and malaria in Sichuan, China." Bulletin of the World Health Organization **73**(3): 321.

Cheng, Q., M. L. Gatton, J. Barnwell, P. Chiodini, J. McCarthy, D. Bell and J. Cunningham (2014). "Plasmodium falciparum parasites lacking histidine-rich protein 2 and 3: a review and recommendations for accurate reporting." Malar J **13**: 283.

Churcher, T. S., R. E. Sinden, N. J. Edwards, I. D. Poulton, T. W. Rampling, P. M. Brock, J. T. Griffin, L. M. Upton, S. E. Zakutansky, K. A. Sala, F. Angrisano, A. V. Hill and A. M. Blagborough (2017). "Probability of Transmission of Malaria from Mosquito to Human Is Regulated by Mosquito Parasite Density in Naive and Vaccinated Hosts." PLoS Pathog **13**(1): e1006108.

Cirimotich, C. M., Y. Dong, A. M. Clayton, S. L. Sandiford, J. A. Souza-Neto, M. Mulenga and G. Dimopoulos (2011). "Natural microbe-mediated refractoriness to Plasmodium infection in Anopheles gambiae." Science **332**(6031): 855-858.

Cohen, J. M., B. Moonen, R. W. Snow and D. L. Smith (2010). "How absolute is zero? An evaluation of historical and current definitions of malaria elimination." Malar J **9**: 213.

Collins, W. E. and G. M. Jeffery (2007). "Plasmodium malariae: parasite and disease." Clin Microbiol Rev **20**(4): 579-592.

Conn, J. E., R. C. Wilkerson, M. N. Segura, R. T. de Souza, C. D. Schlichting, R. A. Wirtz and M. M. Povoa (2002). "Emergence of a new neotropical malaria vector facilitated by human migration and changes in land use." Am J Trop Med Hyg **66**(1): 18-22.

Conroy, A. L., C. R. McDonald and K. C. Kain (2012). "Malaria in pregnancy: diagnosing infection and identifying fetal risk." Expert Rev Anti Infect Ther **10**(11): 1331-1342.

Cook, J., H. Reid, J. Iavro, M. Kuwahata, G. Taleo, A. Clements, J. McCarthy, A. Vallely and C. Drakeley (2010). "Using serological measures to monitor changes in malaria transmission in Vanuatu." Malar J **9**: 169.

Cook, J., W. Xu, M. Msellem, M. Vonk, B. Bergstrom, R. Gosling, A. W. Al-Mafazy, P. McElroy, F. Molteni, A. K. Abass, I. Garimo, M. Ramsan, A. Ali, A. Martensson and A. Bjorkman (2015). "Mass screening and treatment on the basis of results of a Plasmodium falciparum-specific rapid diagnostic test did not reduce malaria incidence in Zanzibar." J Infect Dis **211**(9): 1476-1483.

Cornille-Brogger, R., H. M. Mathews, J. Storey, T. S. Ashkar, S. Brogger and L. Molineaux (1978). "Changing patterns in the humoral immune response to malaria before, during, and after the application of control measures: a longitudinal study in the West African savanna." Bull World Health Organ **56**(4): 579-600.

Corran, P., P. Coleman, E. Riley and C. Drakeley (2007). "Serology: a robust indicator of malaria transmission intensity?" Trends Parasitol **23**(12): 575-582.

Cotter, C., H. J. Sturrock, M. S. Hsiang, J. Liu, A. A. Phillips, J. Hwang, C. S. Gueye, N. Fullman, R. D. Gosling and R. G. Feachem (2013). "The changing epidemiology of malaria elimination: new strategies for new challenges." Lancet **382**(9895): 900-911.

Cox-Singh, J., T. M. E. Davis, K.-S. Lee, S. S. G. Shamsul, A. Matusop, S. Ratnam, H. A. Rahman, D. J. Conway and B. Singh (2008). "Plasmodium knowlesi Malaria in Humans Is Widely Distributed and Potentially Life Threatening." Clinical Infectious Diseases **46**(2): 165-171.

Crompton, P. D., B. Traore, K. Kayentao, S. Doumbo, A. Ongoiba, S. A. Diakite, M. A. Krause, D. Doumtabe, Y. Kone, G. Weiss, C. Y. Huang, S. Doumbia, A. Guindo, R. M. Fairhurst, L. H. Miller, S. K. Pierce and O. K. Doumbo (2008). "Sickle cell trait is associated with a delayed onset of malaria: implications for time-to-event analysis in clinical studies of malaria." J Infect Dis **198**(9): 1265-1275.

Cunnington, A. J., M. Njie, S. Correa, E. N. Takem, E. M. Riley and M. Walther (2012). "Prolonged neutrophil dysfunction after Plasmodium falciparum malaria is related to hemolysis and heme oxygenase-1 induction." J Immunol **189**(11): 5336-5346.

Curran, P. J., P. M. Atkinson, G. M. Foody and E. J. Milton (2000). "Linking remote sensing, land cover and disease." Adv Parasitol **47**: 37-80.

Curtis, C. F. (2002). "Should the use of DDT be revived for malaria vector control?" Biomedica **22**(4): 455-461.

Cuzick, J. and R. Edwards (1990). "Spatial clustering for inhomogeneous populations." Journal of the Royal Statistical Society. Series B (Methodological): 73-104.

D'Alessandro, U., A. Leach, C. J. Drakeley, S. Bennett, B. O. Olaleye, G. W. Fegan, M. Jawara, P. Langerock, M. O. George, G. A. Targett and et al. (1995). "Efficacy trial of malaria vaccine SPf66 in Gambian infants." Lancet **346**(8973): 462-467.

De Freece, C., L. Pare Toe, F. Esposito, A. Diabate and G. Favia (2014). "Preliminary assessment of framework conditions for release of genetically modified mosquitoes in Burkina Faso." Int Health **6**(3): 263-265.

Diggle, P. J. and A. G. Chetwynd (1991). "Second-Order Analysis of spatial Clustering for Inhomogeneous Populations." Biometrics **47**: 9.

Doderer-Lang, C., P. S. Atchade, L. Meckert, E. Haar, S. Perrotey, D. Filisetti, A. Aboubacar, A. W. Pfaff, J. Brunet, N. W. Chabi, C. D. Akpovi, L. Anani, A. Bigot, A. Sanni and E. Candolfi (2014). "The ears of the African elephant: unexpected high seroprevalence of Plasmodium ovale and Plasmodium malariae in healthy populations in Western Africa." Malar J **13**: 240.

Dondorp, A., F. Nosten, K. Stepniewska, N. Day and N. White (2005). "Artesunate versus quinine for treatment of severe falciparum malaria: a randomised trial." Lancet **366**(9487): 717-725.

Dondorp, A. M., C. I. Fanello, I. C. Hendriksen, E. Gomes, A. Seni, K. D. Chhaganlal, K. Bojang, R. Olaosebikan, N. Anunobi, K. Maitland, E. Kivaya, T. Agbenyega, S. B. Nguah, J. Evans, S. Gesase, C. Kahabuka, G. Mtove, B. Nadjm, J. Deen, J. Mwanga-Amumpaire, M. Nansumba, C. Karema, N. Umulisa, A. Uwimana, O. A. Mokuolu, O. T. Adedoyin, W. B. Johnson, A. K. Tshefu, M. A. Onyamboko, T. Sakulthaew, W. P. Ngum, K. Silamut, K. Stepniewska, C. J. Woodrow, D. Bethell, B. Wills, M. Oneko, T. E. Peto, L. von Seidlein, N. P. Day and N. J. White (2010). "Artesunate versus quinine in the treatment of severe falciparum malaria in African children (AQUAMAT): an open-label, randomised trial." Lancet **376**(9753): 1647-1657.

Dondorp, A. M., S. J. Lee, M. A. Faiz, S. Mishra, R. Price, E. Tjitra, M. Than, Y. Htut, S. Mohanty, E. B. Yunus, R. Rahman, F. Nosten, N. M. Anstey, N. P. Day and N. J. White (2008). "The relationship between age and the manifestations of and mortality associated with severe malaria." Clin Infect Dis **47**(2): 151-157.

Doolan, D. L., C. Dobano and J. K. Baird (2009). "Acquired immunity to malaria." Clin Microbiol Rev **22**(1): 13-36, Table of Contents.

Drakeley, C. J., P. H. Corran, P. G. Coleman, J. E. Tongren, S. L. McDonald, I. Carneiro, R. Malima, J. Lusingu, A. Manjurano, W. M. Nkya, M. M. Lemnge, J. Cox, H. Reyburn and E. M. Riley (2005). "Estimating medium- and long-term trends in malaria transmission by using serological markers of malaria exposure." Proc Natl Acad Sci U S A **102**(14): 5108-5113.

Dutta, H. M. and A. K. Dutt (1978). "Malarial ecology: a global perspective." Soc Sci Med **12**(2d): 69-84.

Dye, C. and G. Hasibeder (1986). "Population dynamics of mosquito-borne disease: effects of flies which bite some people more frequently than others." Trans R Soc Trop Med Hyg **80**(1): 69-77.

Edozien, J. C., H. M. Gilles and I. O. K. Udeozo "ADULT AND CORD-BLOOD GAMMA-GLOBULIN AND IMMUNITY TO MALARIA IN NIGERIANS." The Lancet **280**(7263): 951-955.

Egger, M., G. Davey Smith, M. Schneider and C. Minder (1997). "Bias in meta-analysis detected by a simple, graphical test." Bmj **315**(7109): 629-634.

Erdman, L. K. and K. C. Kain (2008). "Molecular diagnostic and surveillance tools for global malaria control." Travel Med Infect Dis **6**(1-2): 82-99.

Ernst, K. C., S. O. Adoka, D. O. Kowuor, M. L. Wilson and C. C. John (2006). "Malaria hotspot areas in a highland Kenya site are consistent in epidemic and non-epidemic years and are associated with ecological factors." Malar J **5**: 78.

Fegan, G. W., A. M. Noor, W. S. Akhwale, S. Cousens and R. W. Snow (2007). "Effect of expanded insecticide-treated bednet coverage on child survival in rural Kenya: a longitudinal study." Lancet **370**(9592): 1035-1039.

French, N., J. Nakiyingi, E. Lugada, C. Watera, J. A. Whitworth and C. F. Gilks (2001). "Increasing rates of malarial fever with deteriorating immune status in HIV-1-infected Ugandan adults." Aids **15**(7): 899-906.

Fryauff, D. J., S. Tuti, A. Mardi, S. Masbar, R. Patipelohi, B. Leksana, K. C. Kain, M. J. Bangs, T. L. Richie and J. K. Baird (1998). "Chloroquine-resistant Plasmodium vivax in transmigration settlements of West Kalimantan, Indonesia." Am J Trop Med Hyg **59**(4): 513-518.

Garcia, J. E., A. Puentes and M. E. Patarroyo (2006). "Developmental biology of sporozoite-host interactions in Plasmodium falciparum malaria: implications for vaccine design." Clin Microbiol Rev **19**(4): 686-707.

Garrett-Jones, C. (1964). "THE HUMAN BLOOD INDEX OF MALARIA VECTORS IN RELATION TO EPIDEMIOLOGICAL ASSESSMENT." Bull World Health Organ **30**: 241-261.

Gaudart, J., B. Poudiougou, A. Dicko, S. Ranque, O. Toure, I. Sagara, M. Diallo, S. Diawara, A. Ouattara, M. Diakite and O. K. Doumbo (2006). "Space-time clustering of childhood malaria at the household level: a dynamic cohort in a Mali village." BMC Public Health **6**: 286.

Gething, P. W., D. C. Casey, D. J. Weiss, D. Bisanzio, S. Bhatt, E. Cameron, K. E. Battle, U. Dalrymple, J. Rozier, P. C. Rao, M. J. Kutz, R. M. Barber, C. Huynh, K. A. Shackelford, M. M. Coates, G. Nguyen, M. S. Fraser, R. Kulikoff, H. Wang, M. Naghavi, D. L. Smith, C. J. Murray, S. I. Hay and S. S. Lim (2016). "Mapping Plasmodium falciparum Mortality in Africa between 1990 and 2015." N Engl J Med **375**(25): 2435-2445.

Gething, P. W., I. R. Elyazar, C. L. Moyes, D. L. Smith, K. E. Battle, C. A. Guerra, A. P. Patil, A. J. Tatem, R. E. Howes, M. F. Myers, D. B. George, P. Horby, H. F. Wertheim, R. N. Price, I. Mueller,

J. K. Baird and S. I. Hay (2012). "A long neglected world malaria map: Plasmodium vivax endemicity in 2010." PLoS Negl Trop Dis **6**(9): e1814.

Gething, P. W., A. P. Patil, D. L. Smith, C. A. Guerra, I. R. Elyazar, G. L. Johnston, A. J. Tatem and S. I. Hay (2011). "A new world malaria map: Plasmodium falciparum endemicity in 2010." Malar J **10**: 378.

Getis, A. and J. K. Ord (1992). "The Analysis of Spatial Association by Use of Distance Statistics." Geographical Analysis **24**(3): 189-206.

Ghebreyesus, T. A., M. Haile, K. H. Witten, A. Getachew, A. M. Yohannes, M. Yohannes, H. D. Teklehaimanot, S. W. Lindsay and P. Byass (1999). "Incidence of malaria among children living near dams in northern Ethiopia: community based incidence survey." Bmj **319**(7211): 663-666.

Giardina, F., L. Gosoniu, L. Konate, M. B. Diouf, R. Perry, O. Gaye, O. Faye and P. Vounatsou (2012). "Estimating the burden of malaria in Senegal: Bayesian zero-inflated binomial geostatistical modeling of the MIS 2008 data." PLoS One **7**(3): e32625.

Gosling, R. D., S. Gesase, J. F. Mosha, I. Carneiro, R. Hashim, M. Lemnge, F. W. Mosha, B. Greenwood and D. Chandramohan (2009). "Protective efficacy and safety of three antimalarial regimens for intermittent preventive treatment for malaria in infants: a randomised, double-blind, placebo-controlled trial." Lancet **374**(9700): 1521-1532.

Graham, J. W., A. E. Olchowski and T. D. Gilreath (2007). "How many imputations are really needed? Some practical clarifications of multiple imputation theory." Prevention Science **8**(3): 206-213.

Gray, E. M. and T. J. Bradley (2005). "Physiology of desiccation resistance in Anopheles gambiae and Anopheles arabiensis." Am J Trop Med Hyg **73**(3): 553-559.

Greenwood, B. (2006). "Review: Intermittent preventive treatment–a new approach to the prevention of malaria in children in areas with seasonal malaria transmission." Tropical medicine & international health **11**(7): 983-991.

Greenwood, B. and T. Mutabingwa (2002). "Malaria in 2002." Nature **415**(6872): 670-672.

Guyatt, H. L. and R. W. Snow (2001). "Malaria in pregnancy as an indirect cause of infant mortality in sub-Saharan Africa." Trans R Soc Trop Med Hyg **95**(6): 569-576.

Gwitira, I., A. Murwira, F. M. Zengeya and M. D. Shekede (2018). "Application of GIS to predict malaria hotspots based on Anopheles arabiensis habitat suitability in Southern Africa." International Journal of Applied Earth Observation and Geoinformation **64**: 12-21.

Harris, R. J., M. J. Bradburn, J. J. Deeks, R. M. Harbord, D. G. Altman and J. Sterne (2008). "metan: fixed- and random-effects meta-analysis." Stata Journal **8 (1)**: 3-28.

Hawley, W. A., P. A. Phillips-Howard, F. O. ter Kuile, D. J. Terlouw, J. M. Vulule, M. Ombok, B. L. Nahlen, J. E. Gimnig, S. K. Kariuki, M. S. Kolczak and A. W. Hightower (2003). "Community-wide effects of permethrin-treated bed nets on child mortality and malaria morbidity in western Kenya." Am J Trop Med Hyg **68**(4 Suppl): 121-127.

Hay, S. I. (2000). "An overview of remote sensing and geodesy for epidemiology and public health application." Adv Parasitol **47**: 1-35.

Hay, S. I., C. A. Guerra, A. J. Tatem, P. M. Atkinson and R. W. Snow (2005). "Urbanization, malaria transmission and disease burden in Africa." Nat Rev Microbiol **3**(1): 81-90.

Hendriksen, I. C., J. Ferro, P. Montoya, K. D. Chhaganlal, A. Seni, E. Gomes, K. Silamut, S. J. Lee, M. Lucas, K. Chotivanich, C. I. Fanello, N. P. Day, N. J. White, L. von Seidlein and A. M. Dondorp (2012). "Diagnosis, clinical presentation, and in-hospital mortality of severe malaria in HIV-coinfected children and adults in Mozambique." Clin Infect Dis **55**(8): 1144-1153.

Hendriksen, I. C., G. Mtove, A. J. Pedro, E. Gomes, K. Silamut, S. J. Lee, A. Mwambuli, S. Gesase, H. Reyburn, N. P. Day, N. J. White, L. von Seidlein and A. M. Dondorp (2011). "Evaluation of a PfHRP2 and a pLDH-based rapid diagnostic test for the diagnosis of severe malaria in 2 populations of African children." Clin Infect Dis **52**(9): 1100-1107.

Hennig, B. J., S. A. Unger, B. L. Dondeh, J. Hassan, S. Hawkesworth, L. Jarjou, K. S. Jones, S. E. Moore, H. M. Nabwera, M. Ngum, A. Prentice, B. Sonko, A. M. Prentice and A. J. Fulford (2015). "Cohort Profile: The Kiang West Longitudinal Population Study (KWLPS)-a platform for integrated research and health care provision in rural Gambia." Int J Epidemiol.

Herbreteau, V., G. Salem, M. Souris, J. P. Hugot and J. P. Gonzalez (2007). "Thirty years of use and improvement of remote sensing, applied to epidemiology: from early promises to lasting frustration." Health Place **13**(2): 400-403.

Hermsen, C. C., D. S. Telgt, E. H. Linders, L. A. van de Locht, W. M. Eling, E. J. Mensink and R. W. Sauerwein (2001). "Detection of Plasmodium falciparum malaria parasites in vivo by real-time quantitative PCR." Mol Biochem Parasitol **118**(2): 247-251.

Hogh, B., A. Gamage-Mendis, G. A. Butcher, R. Thompson, K. Begtrup, C. Mendis, S. M. Enosse, M. Dgedge, J. Barreto, W. Eling and R. E. Sinden (1998). "The differing impact of chloroquine and pyrimethamine/sulfadoxine upon the infectivity of malaria species to the mosquito vector." Am J Trop Med Hyg **58**(2): 176-182.

Hopkins, H., W. Kambale, M. R. Kamya, S. G. Staedke, G. Dorsey and P. J. Rosenthal (2007). "Comparison of HRP2- and pLDH-based rapid diagnostic tests for malaria with longitudinal follow-up in Kampala, Uganda." Am J Trop Med Hyg **76**(6): 1092-1097.

Horton, N. J. and S. R. Lipsitz (2001). "Multiple imputation in practice: comparison of software packages for regression models with missing variables." The American Statistician **55**(3): 244-254.

Houze, S., M. D. Boly, J. Le Bras, P. Deloron and J. F. Faucher (2009). "PfHRP2 and PfLDH antigen detection for monitoring the efficacy of artemisinin-based combination therapy (ACT) in the treatment of uncomplicated falciparum malaria." Malar J **8**: 211.

Howard, S. C., J. Omumbo, C. Nevill, E. S. Some, C. A. Donnelly and R. W. Snow (2000). "Evidence for a mass community effect of insecticide-treated bednets on the incidence of malaria on the Kenyan coast." Trans R Soc Trop Med Hyg **94**(4): 357-360.

Howes, R. E., A. P. Patil, F. B. Piel, O. A. Nyangiri, C. W. Kabaria, P. W. Gething, P. A. Zimmerman, C. Barnadas, C. M. Beall, A. Gebremedhin, D. Menard, T. N. Williams, D. J. Weatherall and S. I. Hay (2011). "The global distribution of the Duffy blood group." Nat Commun **2**: 266.

Hsiang, M. S., J. Hwang, A. R. Tao, Y. Liu, A. Bennett, G. D. Shanks, J. Cao, S. P. Kachur, R. G. Feachem, R. D. Gosling and Q. Gao (2013). "Mass drug administration for the control and elimination of Plasmodium vivax malaria: an ecological study from Jiangsu province, China." Malar J **12**: 383.

Ijumba, J. N. and S. W. Lindsay (2001). "Impact of irrigation on malaria in Africa: paddies paradox." Med Vet Entomol **15**(1): 1-11.

Izumi, K., A. Ohkado, K. Uchimura, Y. Murase, Y. Tatsumi, A. Kayebeta, Y. Watanabe and N. Ishikawa (2015). "Detection of Tuberculosis Infection Hotspots Using Activity Spaces Based Spatial Approach in an Urban Tokyo, from 2003 to 2011." PLoS One **10**(9): e0138831.

Jensen, T. P., H. Bukirwa, D. Njama-Meya, D. Francis, M. R. Kamya, P. J. Rosenthal and G. Dorsey (2009). "Use of the slide positivity rate to estimate changes in malaria incidence in a cohort of Ugandan children." Malar J **8**: 213.

Johnston, G. L., D. L. Smith and D. A. Fidock (2013). "Malaria's missing number: calculating the human component of R0 by a within-host mechanistic model of Plasmodium falciparum infection and transmission." PLoS Comput Biol **9**(4): e1003025.

Kamya, M. R., E. Arinaitwe, H. Wanzira, A. Katureebe, C. Barusya, S. P. Kigozi, M. Kilama, A. J. Tatem, P. J. Rosenthal, C. Drakeley, S. W. Lindsay, S. G. Staedke, D. L. Smith, B. Greenhouse and G. Dorsey (2015). "Malaria transmission, infection, and disease at three sites with varied

transmission intensity in Uganda: implications for malaria control." Am J Trop Med Hyg **92**(5): 903-912.

Kangoye, D. T., A. Noor, J. Midega, J. Mwongeli, D. Mkabili, P. Mogeni, C. Kerubo, P. Akoo, J. Mwangangi, C. Drakeley, K. Marsh, P. Bejon and P. Njuguna (2016). "Malaria hotspots defined by clinical malaria, asymptomatic carriage, PCR and vector numbers in a low transmission area on the Kenyan Coast." Malar J **15**: 213.

Karagiannis-Voules, D. A., R. G. Scholte, L. H. Guimaraes, J. Utzinger and P. Vounatsou (2013). "Bayesian geostatistical modeling of leishmaniasis incidence in Brazil." PLoS Negl Trop Dis **7**(5): e2213.

Karema, C., M. W. Aregawi, A. Rukundo, A. Kabayiza, M. Mulindahabi, I. S. Fall, K. Gausi, R. O. Williams, M. Lynch, R. Cibulskis, N. Fidele, J. P. Nyemazi, D. Ngamije, I. Umulisa, R. Newman and A. Binagwaho (2012). "Trends in malaria cases, hospital admissions and deaths following scale-up of anti-malarial interventions, 2000-2010, Rwanda." Malar J **11**: 236.

Kasasa, S., V. Asoala, L. Gosoniu, F. Anto, M. Adjuik, C. Tindana, T. Smith, S. Owusu-Agyei and P. Vounatsou (2013). "Spatio-temporal malaria transmission patterns in Navrongo demographic surveillance site, northern Ghana." Malar J **12**: 63.

Katz, J., A. C. Lee, N. Kozuki, J. E. Lawn, S. Cousens, H. Blencowe, M. Ezzati, Z. A. Bhutta, T. Marchant, B. A. Willey, L. Adair, F. Barros, A. H. Baqui, P. Christian, W. Fawzi, R. Gonzalez, J. Humphrey, L. Huybregts, P. Kolsteren, A. Mongkolchati, L. C. Mullany, R. Ndyomugyenyi, J. K. Nien, D. Osrin, D. Roberfroid, A. Sania, C. Schmiegelow, M. F. Silveira, J. Tielsch, A. Vaidya, S. C. Velaphi, C. G. Victora, D. Watson-Jones and R. E. Black (2013). "Mortality risk in preterm and small-for-gestational-age infants in low-income and middle-income countries: a pooled country analysis." Lancet **382**(9890): 417-425.

Kayentao, K., P. Garner, A. M. van Eijk, I. Naidoo, C. Roper, A. Mulokozi, J. R. MacArthur, M. Luntamo, P. Ashorn, O. K. Doumbo and F. O. ter Kuile (2013). "Intermittent preventive therapy

for malaria during pregnancy using 2 vs 3 or more doses of sulfadoxine-pyrimethamine and risk of low birth weight in Africa: systematic review and meta-analysis." Jama **309**(6): 594-604.

Kelly-Hope, L. A. and F. E. McKenzie (2009). "The multiplicity of malaria transmission: a review of entomological inoculation rate measurements and methods across sub-Saharan Africa." Malar J **8**: 19.

Kelsall, J. E. and P. J. Diggle (1995). "Non-parametric estimation of spatial variation in relative risk." Statistics in medicine **14**(21-22): 2335-2342.

Kidane, G. and R. H. Morrow (2000). "Teaching mothers to provide home treatment of malaria in Tigray, Ethiopia: a randomised trial." Lancet **356**(9229): 550-555.

Kilama, M., D. L. Smith, R. Hutchinson, R. Kigozi, A. Yeka, G. Lavoy, M. R. Kamya, S. G. Staedke, M. J. Donnelly, C. Drakeley, B. Greenhouse, G. Dorsey and S. W. Lindsay (2014). "Estimating the annual entomological inoculation rate for Plasmodium falciparum transmitted by Anopheles gambiae s.l. using three sampling methods in three sites in Uganda." Malar J **13**: 111.

Killeen, G. F., U. Fillinger and B. G. Knols (2002). "Advantages of larval control for African malaria vectors: low mobility and behavioural responsiveness of immature mosquito stages allow high effective coverage." Malar J **1**: 8.

Killeen, G. F., A. Ross and T. Smith (2006). "Infectiousness of malaria-endemic human populations to vectors." Am J Trop Med Hyg **75**(2 Suppl): 38-45.

Kim, D., K. Fedak and R. Kramer (2012). "Reduction of malaria prevalence by indoor residual spraying: a meta-regression analysis." Am J Trop Med Hyg **87**(1): 117-124.

Kirby, M. J. and S. W. Lindsay (2004). "Responses of adult mosquitoes of two sibling species, Anopheles arabiensis and A. gambiae s.s. (Diptera: Culicidae), to high temperatures." Bull Entomol Res **94**(5): 441-448.

KNBS (2009). Kenya Census 2009, Kilifi County.

Koepfli, C., S. Schoepflin, M. Bretscher, E. Lin, B. Kiniboro, P. A. Zimmerman, P. Siba, T. A. Smith, I. Mueller and I. Felger (2011). "How much remains undetected? Probability of molecular detection of human Plasmodia in the field." PLoS One **6**(4): e19010.

Kolaczinski, K., J. Kolaczinski, A. Kilian and S. Meek (2007). "Extension of indoor residual spraying for malaria control into high transmission settings in Africa." Trans R Soc Trop Med Hyg **101**(9): 852-853.

Kreuels, B., R. Kobbe, S. Adjei, C. Kreuzberg, C. von Reden, K. Bater, S. Klug, W. Busch, O. Adjei and J. May (2008). "Spatial variation of malaria incidence in young children from a geographically homogeneous area with high endemicity." J Infect Dis **197**(1): 85-93.

Kulkarni, M. A., R. E. Desrochers and J. T. Kerr (2010). "High resolution niche models of malaria vectors in northern Tanzania: a new capacity to predict malaria risk?" PLoS One **5**(2): e9396.

Kulldorff, M. (1997). "A spatial-scan statistic." Comput Stat: Theory Methods **26**.

Kulldorff, M. and U. Hjalmars (1999). "The Knox Method and Other Tests for Space-Time Interaction." Biometrics **55**(2): 544-552.

Kunene, S., A. A. Phillips, R. D. Gosling, D. Kandula and J. M. Novotny (2011). "A national policy for malaria elimination in Swaziland: a first for sub-Saharan Africa." Malar J **10**: 313.

Lawn, J. E., H. Blencowe, P. Waiswa, A. Amouzou, C. Mathers, D. Hogan, V. Flenady, J. F. Froen, Z. U. Qureshi, C. Calderwood, S. Shiekh, F. B. Jassir, D. You, E. M. McClure, M. Mathai and S. Cousens (2016). "Stillbirths: rates, risk factors, and acceleration towards 2030." Lancet **387**(10018): 587-603.

Lengeler, C. (2000). "Insecticide-treated bednets and curtains for preventing malaria." Cochrane Database Syst Rev(2): Cd000363.

Levy, M. Z., V. Kawai, N. M. Bowman, L. A. Waller, L. Cabrera, V. V. Pinedo-Cancino, A. E. Seitz, F. J. Steurer, J. G. Cornejo del Carpio, E. Cordova-Benzaquen, J. H. Maguire, R. H. Gilman and C. Bern (2007). "Targeted screening strategies to detect Trypanosoma cruzi infection in children." PLoS Negl Trop Dis **1**(3): e103.

Lim, C. S., J. K. Yoon, E. A. Chang, I. B. Suh, S. S. An, K. H. Lee, J. T. Chung and Y. C. Tockgo (2005). "Seroprevalence to the circumsporozoite protein peptide antigen of Plasmodium vivax in Korean children." Microbiol Immunol **49**(6): 521-527.

Lim, S. S., N. Fullman, A. Stokes, N. Ravishankar, F. Masiye, C. J. Murray and E. Gakidou (2011). "Net benefits: a multicountry analysis of observational data examining associations between insecticide-treated mosquito nets and health outcomes." PLoS Med **8**(9): e1001091.

Lindblade, K. A., T. P. Eisele, J. E. Gimnig, J. A. Alaii, F. Odhiambo, F. O. ter Kuile, W. A. Hawley, K. A. Wannemuehler, P. A. Phillips-Howard, D. H. Rosen, B. L. Nahlen, D. J. Terlouw, K. Adazu, J. M. Vulule and L. Slutsker (2004). "Sustainability of reductions in malaria transmission and infant mortality in western Kenya with use of insecticide-treated bednets: 4 to 6 years of follow-up." Jama **291**(21): 2571-2580.

Lindblade, K. A., L. Steinhardt, A. Samuels, S. P. Kachur and L. Slutsker (2013). "The silent threat: asymptomatic parasitemia and malaria transmission." Expert Rev Anti Infect Ther **11**(6): 623-639.

Lindblade, K. A., E. D. Walker, A. W. Onapa, J. Katungu and M. L. Wilson (1999). "Highland malaria in Uganda: prospective analysis of an epidemic associated with El Nino." Trans R Soc Trop Med Hyg **93**(5): 480-487.

Mabaso, M. L., P. Vounatsou, S. Midzi, J. Da Silva and T. Smith (2006). "Spatio-temporal analysis of the role of climate in inter-annual variation of malaria incidence in Zimbabwe." Int J Health Geogr **5**: 20.

Mabey, D. C., A. Brown and B. M. Greenwood (1987). "Plasmodium falciparum malaria and Salmonella infections in Gambian children." J Infect Dis **155**(6): 1319-1321.

Mackenzie, G., S. J. Ceesay, P. C. Hill, M. Walther, K. A. Bojang, J. Satoguina, G. Enwere, U. D'Alessandro, D. Saha, U. N. Ikumapayi, T. O'Dempsey, D. C. Mabey, T. Corrah, D. J. Conway, R. A. Adegbola and B. M. Greenwood (2010). "A decline in the incidence of invasive non-typhoidal Salmonella infection in The Gambia temporally associated with a decline in malaria infection." PLoS One **5**(5): e10568.

Mackinnon, M. J., T. W. Mwangi, R. W. Snow, K. Marsh and T. N. Williams (2005). "Heritability of malaria in Africa." PLoS Med **2**(12): e340.

Maitland, K., S. Kiguli, R. O. Opoka, C. Engoru, P. Olupot-Olupot, S. O. Akech, R. Nyeko, G. Mtove, H. Reyburn, T. Lang, B. Brent, J. A. Evans, J. K. Tibenderana, J. Crawley, E. C. Russell, M. Levin, A. G. Babiker and D. M. Gibb (2011). "Mortality after fluid bolus in African children with severe infection." N Engl J Med **364**(26): 2483-2495.

malERA (2011). "A research agenda for malaria eradication: diagnoses and diagnostics." PLoS Med **8**(1): e1000396.

Manzi, F., J. Schellenberg, Y. Hamis, A. K. Mushi, K. Shirima, A. Mwita, A. Simba, N. Rusibamayila, M. Kitambi, M. Tanner, P. Alonso, H. Mshinda and D. Schellenberg (2009). "Intermittent preventive treatment for malaria and anaemia control in Tanzanian infants; the development and implementation of a public health strategy." Trans R Soc Trop Med Hyg **103**(1): 79-86.

Marsh, K., D. Forster, C. Waruiru, I. Mwangi, M. Winstanley, V. Marsh, C. Newton, P. Winstanley, P. Warn, N. Peshu and et al. (1995). "Indicators of life-threatening malaria in African children." N Engl J Med **332**(21): 1399-1404.

Marsh, K. and S. Kinyanjui (2006). "Immune effector mechanisms in malaria." Parasite Immunol **28**(1-2): 51-60.

Marsh, V. M., W. M. Mutemi, J. Muturi, A. Haaland, W. M. Watkins, G. Otieno and K. Marsh (1999). "Changing home treatment of childhood fevers by training shop keepers in rural Kenya." Trop Med Int Health **4**(5): 383-389.

Martens, P. and L. Hall (2000). "Malaria on the move: human population movement and malaria transmission." Emerg Infect Dis **6**(2): 103-109.

Martensson, A., J. Stromberg, C. Sisowath, M. I. Msellem, J. P. Gil, S. M. Montgomery, P. Olliaro, A. S. Ali and A. Bjorkman (2005). "Efficacy of artesunate plus amodiaquine versus that of artemether-lumefantrine for the treatment of uncomplicated childhood Plasmodium falciparum malaria in Zanzibar, Tanzania." Clin Infect Dis **41**(8): 1079-1086.

McCoy, D., G. Kembhavi, J. Patel and A. Luintel (2009). "The Bill & Melinda Gates Foundation's grant-making programme for global health." Lancet **373**(9675): 1645-1653.

Menard, D., C. Barnadas, C. Bouchier, C. Henry-Halldin, L. R. Gray, A. Ratsimbasoa, V. Thonier, J. F. Carod, O. Domarle, Y. Colin, O. Bertrand, J. Picot, C. L. King, B. T. Grimberg, O. Mercereau-Puijalon and P. A. Zimmerman (2010). "Plasmodium vivax clinical malaria is commonly observed in Duffy-negative Malagasy people." Proc Natl Acad Sci U S A **107**(13): 5967-5971.

Mendes, C., F. Dias, J. Figueiredo, V. G. Mora, J. Cano, B. de Sousa, V. E. do Rosario, A. Benito, P. Berzosa and A. P. Arez (2011). "Duffy negative antigen is no longer a barrier to Plasmodium vivax--molecular evidences from the African West Coast (Angola and Equatorial Guinea)." PLoS Negl Trop Dis **5**(6): e1192.

Mendis, K., B. J. Sina, P. Marchesini and R. Carter (2001). "The neglected burden of Plasmodium vivax malaria." Am J Trop Med Hyg **64**(1-2 Suppl): 97-106.

Midega, J. T., D. L. Smith, A. Olotu, J. M. Mwangangi, J. G. Nzovu, J. Wambua, G. Nyangweso, C. M. Mbogo, G. K. Christophides, K. Marsh and P. Bejon (2012). "Wind direction and proximity to larval sites determines malaria risk in Kilifi District in Kenya." Nat Commun **3**: 674.

Midekisa, A., G. Senay, G. M. Henebry, P. Semuniguse and M. C. Wimberly (2012). "Remote sensing-based time series models for malaria early warning in the highlands of Ethiopia." Malar J **11**: 165.

Miller, L. H., S. J. Mason, D. F. Clyde and M. H. McGinniss (1976). "The resistance factor to Plasmodium vivax in blacks. The Duffy-blood-group genotype, FyFy." N Engl J Med **295**(6): 302-304.

Miller, L. H., S. J. Mason, J. A. Dvorak, M. H. McGinniss and I. K. Rothman (1975). "Erythrocyte receptors for (Plasmodium knowlesi) malaria: Duffy blood group determinants." Science **189**(4202): 561-563.

Minakawa, N., G. Sonye and G. Yan (2005). "Relationships between occurrence of Anopheles gambiae s.l. (Diptera: Culicidae) and size and stability of larval habitats." J Med Entomol **42**(3): 295-300.

Mittlböck, M. and M. Schemper (1996). "Explained variation for logistic regression." Statistics in medicine **15**(19): 1987-1997.

Mmbando, B. P., L. S. Vestergaard, A. Y. Kitua, M. M. Lemnge, T. G. Theander and J. P. Lusingu (2010). "A progressive declining in the burden of malaria in north-eastern Tanzania." Malar J **9**: 216.

Mogeni, P., I. Omedo, C. Nyundo, A. Kamau, A. Noor and P. Bejon (2017a). "Effect of transmission intensity on hotspots and micro-epidemiology of malaria in sub-Saharan Africa." BMC Med **15**(1): 121.

Mogeni, P., T. N. Williams, G. Fegan, C. Nyundo, E. Bauni, K. Mwai, I. Omedo, P. Njuguna, C. R. Newton, F. Osier, J. A. Berkley, L. L. Hammitt, B. Lowe, G. Mwambingu, K. Awuondo, N. Mturi, N. Peshu, R. W. Snow, A. Noor, K. Marsh and P. Bejon (2016). "Age, Spatial, and Temporal Variations in Hospital Admissions with Malaria in Kilifi County, Kenya: A 25-Year Longitudinal Observational Study." PLoS Med **13**(6): e1002047.

Mogeni, P., T. N. Williams, I. Omedo, D. Kimani, J. M. Ngoi, J. Mwacharo, R. Morter, C. Nyundo, J. Wambua and G. Nyangweso (2017b). "Detecting Malaria Hotspots: a comparison between RDT, Microscopy and Polymerase Chain Reaction." The Journal of Infectious Diseases.

Molineaux, L. G., G. (1980). "The Garki Project." Geneva: World Health Organization.

Moody, A. (2002). "Rapid diagnostic tests for malaria parasites." Clin Microbiol Rev **15**(1): 66-78.

Moonen, B., J. M. Cohen, R. W. Snow, L. Slutsker, C. Drakeley, D. L. Smith, R. R. Abeyasinghe, M. H. Rodriguez, R. Maharaj, M. Tanner and G. Targett (2010). "Operational strategies to achieve and maintain malaria elimination." Lancet **376**(9752): 1592-1603.

Mosha, J. F., H. J. Sturrock, B. Greenhouse, B. Greenwood, C. J. Sutherland, N. Gadalla, S. Atwal, C. Drakeley, G. Kibiki, T. Bousema, D. Chandramohan and R. Gosling (2013). "Epidemiology of subpatent Plasmodium falciparum infection: implications for detection of hotspots with imperfect diagnostics." Malar J **12**: 221.

Moyes, C. L., A. J. Henry, N. Golding, Z. Huang, B. Singh, J. K. Baird, P. N. Newton, M. Huffman, K. A. Duda, C. J. Drakeley, I. R. Elyazar, N. M. Anstey, Q. Chen, Z. Zommers, S. Bhatt, P. W. Gething and S. I. Hay (2014). "Defining the geographical range of the Plasmodium knowlesi reservoir." PLoS Negl Trop Dis **8**(3): e2780.

Mueller, I., S. Schoepflin, T. A. Smith, K. L. Benton, M. T. Bretscher, E. Lin, B. Kiniboro, P. A. Zimmerman, T. P. Speed, P. Siba and I. Felger (2012). "Force of infection is key to understanding the epidemiology of Plasmodium falciparum malaria in Papua New Guinean children." Proc Natl Acad Sci U S A **109**(25): 10030-10035.

Mueller, I., P. A. Zimmerman and J. C. Reeder (2007). "Plasmodium malariae and Plasmodium ovale--the "bashful" malaria parasites." Trends Parasitol **23**(6): 278-283.

Mulrow, C. D. (1994). "Rationale for systematic reviews." Bmj **309**(6954): 597-599.

Murray, C. J., R. Lozano, A. D. Flaxman, P. Serina, D. Phillips, A. Stewart, S. L. James, A. Vahdatpour, C. Atkinson, M. K. Freeman, S. L. Ohno, R. Black, S. M. Ali, A. H. Baqui, L. Dandona, E. Dantzer, G. L. Darmstadt, V. Das, U. Dhingra, A. Dutta, W. Fawzi, S. Gomez, B. Hernandez, R. Joshi, H. D. Kalter, A. Kumar, V. Kumar, M. Lucero, S. Mehta, B. Neal, D. Praveen, Z. Premji, D. Ramirez-Villalobos, H. Remolador, I. Riley, M. Romero, M. Said, D. Sanvictores, S. Sazawal, V. Tallo and A. D. Lopez (2014). "Using verbal autopsy to measure causes of death: the comparative performance of existing methods." BMC Med **12**: 5.

Murray, C. J., L. C. Rosenfeld, S. S. Lim, K. G. Andrews, K. J. Foreman, D. Haring, N. Fullman, M. Naghavi, R. Lozano and A. D. Lopez (2012). "Global malaria mortality between 1980 and 2010: a systematic analysis." Lancet **379**(9814): 413-431.

Murray, C. K., R. A. Gasser, Jr., A. J. Magill and R. S. Miller (2008). "Update on rapid diagnostic testing for malaria." Clin Microbiol Rev **21**(1): 97-110.

Murungi, L. M., K. Sonden, D. Odera, L. B. Oduor, F. Guleid, I. N. Nkumama, M. Otiende, D. T. Kangoye, G. Fegan, A. Farnert, K. Marsh and F. H. Osier (2017). "Cord blood IgG and the risk of severe Plasmodium falciparum malaria in the first year of life." Int J Parasitol **47**(2-3): 153-162.

Mwangi, T. W., A. Ross, R. W. Snow and K. Marsh (2005). "Case definitions of clinical malaria under different transmission conditions in Kilifi District, Kenya." J Infect Dis **191**(11): 1932-1939.

Najera, J. A., M. Gonzalez-Silva and P. L. Alonso (2011). "Some lessons for the future from the Global Malaria Eradication Programme (1955-1969)." PLoS Med **8**(1): e1000412.

Nankabirwa, J., D. Zurovac, J. N. Njogu, J. B. Rwakimari, H. Counihan, R. W. Snow and J. K. Tibenderana (2009). "Malaria misdiagnosis in Uganda--implications for policy change." Malar J **8**: 66.

Nevill, C. G., E. S. Some, V. O. Mung'ala, W. Mutemi, L. New, K. Marsh, C. Lengeler and R. W. Snow (1996). "Insecticide-treated bednets reduce mortality and severe morbidity from malaria among children on the Kenyan coast." Trop Med Int Health **1**(2): 139-146.

Newby, G., J. Hwang, K. Koita, I. Chen, B. Greenwood, L. von Seidlein, G. D. Shanks, L. Slutsker, S. P. Kachur, J. Wegbreit, M. M. Ippolito, E. Poirot and R. Gosling (2015). "Review of mass drug administration for malaria and its operational challenges." Am J Trop Med Hyg **93**(1): 125-134.

Nkumama, I. N., W. P. O'Meara and F. H. Osier (2017). "Changes in Malaria Epidemiology in Africa and New Challenges for Elimination." Trends Parasitol **33**(2): 128-140.

Noor, A. M., A. A. Amin, W. S. Akhwale and R. W. Snow (2007). "Increasing coverage and decreasing inequity in insecticide-treated bed net use among rural Kenyan children." PLoS Med **4**(8): e255.

Noor, A. M., P. W. Gething, V. A. Alegana, A. P. Patil, S. I. Hay, E. Muchiri, E. Juma and R. W. Snow (2009a). "The risks of malaria infection in Kenya in 2009." BMC Infect Dis **9**: 180.

Noor, A. M., D. K. Kinyoki, C. W. Mundia, C. W. Kabaria, J. W. Mutua, V. A. Alegana, I. S. Fall and R. W. Snow (2014). "The changing risk of Plasmodium falciparum malaria infection in Africa: 2000-10: a spatial and temporal analysis of transmission intensity." Lancet **383**(9930): 1739-1747.

Noor, A. M., V. C. Kirui, S. J. Brooker and R. W. Snow (2009b). "The use of insecticide treated nets by age: implications for universal coverage in Africa." BMC Public Health **9**: 369.

Noor, A. M., J. A. Omumbo, A. A. Amin, D. Zurovac and R. W. Snow (2006). "Wealth, mother's education and physical access as determinants of retail sector net use in rural Kenya." Malar J **5**: 5.

O'Meara, W. P., P. Bejon, T. W. Mwangi, E. A. Okiro, N. Peshu, R. W. Snow, C. R. Newton and K. Marsh (2008). "Effect of a fall in malaria transmission on morbidity and mortality in Kilifi, Kenya." Lancet **372**(9649): 1555-1562.

O'Meara, W. P., W. E. Collins and F. E. McKenzie (2007). "Parasite prevalence: a static measure of dynamic infections." Am J Trop Med Hyg **77**(2): 246-249.

O'Meara, W. P., J. N. Mangeni, R. Steketee and B. Greenwood (2010). "Changes in the burden of malaria in sub-Saharan Africa." The Lancet Infectious Diseases **10**(8): 545-555.

Ogwang, C., D. Kimani, N. J. Edwards, R. Roberts, J. Mwacharo, G. Bowyer, C. Bliss, S. H. Hodgson, P. Njuguna, N. K. Viebig, A. Nicosia, E. Gitau, S. Douglas, J. Illingworth, K. Marsh, A. Lawrie, E. B. Imoukhuede, K. Ewer, B. C. Urban, S. H. AV and P. Bejon (2015). "Prime-boost vaccination with chimpanzee adenovirus and modified vaccinia Ankara encoding TRAP provides partial protection against Plasmodium falciparum infection in Kenyan adults." Sci Transl Med **7**(286): 286re285.

Okell, L. C., T. Bousema, J. T. Griffin, A. L. Ouedraogo, A. C. Ghani and C. J. Drakeley (2012). "Factors determining the occurrence of submicroscopic malaria infections and their relevance for control." Nat Commun **3**: 1237.

Okell, L. C., A. C. Ghani, E. Lyons and C. J. Drakeley (2009). "Submicroscopic infection in Plasmodium falciparum-endemic populations: a systematic review and meta-analysis." J Infect Dis **200**(10): 1509-1517.

Okell, L. C., J. T. Griffin, I. Kleinschmidt, T. D. Hollingsworth, T. S. Churcher, M. J. White, T. Bousema, C. J. Drakeley and A. C. Ghani (2011). "The potential contribution of mass treatment to the control of Plasmodium falciparum malaria." PLoS One **6**(5): e20179.

Okiro, E. A., A. Al-Taiar, H. Reyburn, R. Idro, J. A. Berkley and R. W. Snow (2009). "Age patterns of severe paediatric malaria and their relationship to Plasmodium falciparum transmission intensity." Malar J **8**: 4.

Okiro, E. A., D. Bitira, G. Mbabazi, A. Mpimbaza, V. A. Alegana, A. O. Talisuna and R. W. Snow (2011). "Increasing malaria hospital admissions in Uganda between 1999 and 2009." BMC Med **9**: 37.

Okiro, E. A., S. I. Hay, P. W. Gikandi, S. K. Sharif, A. M. Noor, N. Peshu, K. Marsh and R. W. Snow (2007). "The decline in paediatric malaria admissions on the coast of Kenya." Malar J **6**: 151.

Olotu, A., G. Fegan, J. Wambua, G. Nyangweso, A. Leach, M. Lievens, D. C. Kaslow, P. Njuguna, K. Marsh and P. Bejon (2016). "Seven-Year Efficacy of RTS,S/AS01 Malaria Vaccine among Young African Children." N Engl J Med **374**(26): 2519-2529.

Olson, S. H., R. Gangnon, G. A. Silveira and J. A. Patz (2010). "Deforestation and malaria in Mancio Lima County, Brazil." Emerg Infect Dis **16**(7): 1108-1115.

Omumbo, J. A., C. A. Guerra, S. I. Hay and R. W. Snow (2005). "The influence of urbanisation on measures of Plasmodium falciparum infection prevalence in East Africa." Acta Trop **93**(1): 11-21.

Openshaw, S., M. Charlton, C. Wymer and A. Craft (1987). "A mark 1 geographical analysis machine for the automated analysis of point data sets." International Journal of Geographical Information System **1**(4): 335-358.

Ostfeld, R. S., G. E. Glass and F. Keesing (2005). "Spatial epidemiology: an emerging (or re-emerging) discipline." Trends Ecol Evol **20**(6): 328-336.

Packard, R. M. and P. Gadehla (1997). "A land filled with mosquitoes: Fred L. Soper, the Rockefeller Foundation, and the anopheles gambiae invasion of Brazil." Med Anthropol **17**(3): 215-238.

Pambana, E. (1963). "A textbook of malaria eradication." London:Oxford university Press.

Patz, J. A., T. K. Graczyk, N. Geller and A. Y. Vittor (2000). "Effects of environmental change on emerging parasitic diseases." Int J Parasitol **30**(12-13): 1395-1405.

Patz, J. A. and S. H. Olson (2006). "Malaria risk and temperature: influences from global climate change and local land use practices." Proc Natl Acad Sci U S A **103**(15): 5635-5636.

Pemberton-Ross, P., T. A. Smith, E. M. Hodel, K. Kay and M. A. Penny (2015). "Age-shifting in malaria incidence as a result of induced immunological deficit: a simulation study." Malar J **14**: 287.

Pitt, S., B. E. Pearcy, R. H. Stevens, A. Sharipov, K. Satarov and N. Banatvala (1998). "War in Tajikistan and re-emergence of Plasmodium falciparum." Lancet **352**(9136): 1279.

Pulford, J., M. W. Hetzel, M. Bryant, P. M. Siba and I. Mueller (2011). "Reported reasons for not using a mosquito net when one is available: a review of the published literature." Malar J **10**: 83.

Pullan, R. L., H. Bukirwa, S. G. Staedke, R. W. Snow and S. Brooker (2010). "Plasmodium infection and its risk factors in eastern Uganda." Malar J **9**: 2.

Pullan, R. L., H. J. Sturrock, R. J. Soares Magalhaes, A. C. Clements and S. J. Brooker (2012). "Spatial parasite ecology and epidemiology: a review of methods and applications." Parasitology **139**(14): 1870-1887.

Ramasamy, R. (2014). "Zoonotic malaria - global overview and research and policy needs." Front Public Health **2**: 123.

Reyburn, H., R. Mbatia, C. Drakeley, J. Bruce, I. Carneiro, R. Olomi, J. Cox, W. M. Nkya, M. Lemnge, B. M. Greenwood and E. M. Riley (2005). "Association of transmission intensity and age with clinical manifestations and case fatality of severe Plasmodium falciparum malaria." Jama **293**(12): 1461-1470.

Reyburn, H., R. Mbatia, C. Drakeley, I. Carneiro, E. Mwakasungula, O. Mwerinde, K. Saganda, J. Shao, A. Kitua, R. Olomi, B. M. Greenwood and C. J. Whitty (2004). "Overdiagnosis of malaria in patients with severe febrile illness in Tanzania: a prospective study." Bmj **329**(7476): 1212.

Riedel, N., P. Vounatsou, J. M. Miller, L. Gosoniu, E. Chizema-Kawesha, V. Mukonka and R. W. Steketee (2010). "Geographical patterns and predictors of malaria risk in Zambia: Bayesian

geostatistical modelling of the 2006 Zambia national malaria indicator survey (ZMIS)." Malar J **9**: 37.

Ripley, B. D. (1976). "The second-order analysis of stationary point processes." Journal of applied probability **13**(2): 255-266.

Ripley, B. D. (1977). "Modelling Spatial Patterns." Journal of the Royal Statistical Society **39**(2): 40.

Roberts, D. R., S. Manguin and J. Mouchet (2000). "DDT house spraying and re-emerging malaria." Lancet **356**(9226): 330-332.

Roberts, L. and M. Enserink (2007). "Malaria. Did they really say ... eradication?" Science **318**(5856): 1544-1545.

Rogers, D. J., S. E. Randolph, R. W. Snow and S. I. Hay (2002). "Satellite imagery in the study and forecast of malaria." Nature **415**(6872): 710-715.

Royston, P. and W. Sauerbrei (2003). "Stability of multivariable fractional polynomial models with selection of variables and transformations: a bootstrap investigation." Stat Med **22**(4): 639-659.

RTS, S. (2015). "Efficacy and safety of RTS,S/AS01 malaria vaccine with or without a booster dose in infants and children in Africa: final results of a phase 3, individually randomised, controlled trial." Lancet **386**(9988): 31-45.

Rubin, D. B. (2004). Multiple imputation for nonresponse in surveys, John Wiley & Sons.

Sabbatani, S., S. Fiorino and R. Manfredi (2010). "The emerging of the fifth malaria parasite (Plasmodium knowlesi): a public health concern?" Brazilian Journal of Infectious Diseases **14**: 299-309.

Sauerbrei, W., C. Meier-Hirmer, A. Benner and P. Royston (2006). "Multivariable regression model building by using fractional polynomials: description of SAS, STATA and R programs." Computational Statistics & Data Analysis **50**(12): 3464-3485.

Sauerbrei, W. and P. Royston (1999). "Building multivariable prognostic and diagnostic models: transformation of the predictors by using fractional polynomials." Journal of the Royal Statistical Society: Series A (Statistics in Society) **162**(1): 71-94.

Sauerbrei, W., P. Royston and H. Binder (2007). "Selection of important variables and determination of functional form for continuous predictors in multivariable model building." Statistics in medicine **26**(30): 5512-5528.

Scholte, R. G., L. Gosoniu, J. B. Malone, F. Chammartin, J. Utzinger and P. Vounatsou (2014). "Predictive risk mapping of schistosomiasis in Brazil using Bayesian geostatistical models." Acta Trop **132**: 57-63.

Schultz, L. J., R. W. Steketee, A. Macheso, P. Kazembe, L. Chitsulo and J. J. Wirima (1994). "The efficacy of antimalarial regimens containing sulfadoxine-pyrimethamine and/or chloroquine in preventing peripheral and placental Plasmodium falciparum infection among pregnant women in Malawi." Am J Trop Med Hyg **51**(5): 515-522.

Scott, J. A., E. Bauni, J. C. Moisi, J. Ojal, H. Gatakaa, C. Nyundo, C. S. Molyneux, F. Kombe, B. Tsofa, K. Marsh, N. Peshu and T. N. Williams (2012). "Profile: The Kilifi Health and Demographic Surveillance System (KHDSS)." Int J Epidemiol **41**(3): 650-657.

Scott, J. A., J. A. Berkley, I. Mwangi, L. Ochola, S. Uyoga, A. Macharia, C. Ndila, B. S. Lowe, S. Mwarumba, E. Bauni, K. Marsh and T. N. Williams (2011). "Relation between falciparum malaria and bacteraemia in Kenyan children: a population-based, case-control study and a longitudinal study." Lancet **378**(9799): 1316-1323.

Sheehy, S. H., C. J. Duncan, S. C. Elias, P. Choudhary, S. Biswas, F. D. Halstead, K. A. Collins, N. J. Edwards, A. D. Douglas, N. A. Anagnostou, K. J. Ewer, T. Havelock, T. Mahungu, C. M. Bliss, K.

Miura, I. D. Poulton, P. J. Lillie, R. D. Antrobus, E. Berrie, S. Moyle, K. Gantlett, S. Colloca, R. Cortese, C. A. Long, R. E. Sinden, S. C. Gilbert, A. M. Lawrie, T. Doherty, S. N. Faust, A. Nicosia, A. V. Hill and S. J. Draper (2012). "ChAd63-MVA-vectored blood-stage malaria vaccines targeting MSP1 and AMA1: assessment of efficacy against mosquito bite challenge in humans." Mol Ther **20**(12): 2355-2368.

SHILILU, J., T. GHEBREMESKEL, S. MENGISTU, H. FEKADU, M. ZEROM, C. MBOGO, J. GITHURE, R. NOVAK, E. BRANTLY and J. C. BEIER (2003). "HIGH SEASONAL VARIATION IN ENTOMOLOGIC INOCULATION RATES IN ERITREA, A SEMI-ARID REGION OF UNSTABLE MALARIA IN AFRICA." The American Journal of Tropical Medicine and Hygiene **69**(6): 607-613.

Shililu, J. I., W. B. Grueber, C. M. Mbogo, J. I. Githure, L. M. Riddiford and J. C. Beier (2004). "Development and survival of Anopheles gambiae eggs in drying soil: influence of the rate of drying, egg age, and soil type." J Am Mosq Control Assoc **20**(3): 243-247.

Shousha, A. T. (1948). "Species-eradication: The Eradication of Anopheles gambioe from Upper Egypt, 1942-1945." Bull World Health Organ **1**(2): 309-352.

Shretta, R., J. Omumbo, B. Rapuoda and R. W. Snow (2000). "Using evidence to change antimalarial drug policy in Kenya." Trop Med Int Health **5**(11): 755-764.

Singh, B., L. Kim Sung, A. Matusop, A. Radhakrishnan, S. S. Shamsul, J. Cox-Singh, A. Thomas and D. J. Conway (2004). "A large focus of naturally acquired Plasmodium knowlesi infections in human beings." Lancet **363**(9414): 1017-1024.

Sinka, M. E., M. J. Bangs, S. Manguin, Y. Rubio-Palis, T. Chareonviriyaphap, M. Coetzee, C. M. Mbogo, J. Hemingway, A. P. Patil, W. H. Temperley, P. W. Gething, C. W. Kabaria, T. R. Burkot, R. E. Harbach and S. I. Hay (2012). "A global map of dominant malaria vectors." Parasit Vectors **5**: 69.

Sissoko, M. S., L. L. van den Hoogen, Y. Samake, A. Tapily, A. Z. Diarra, M. Coulibaly, M. Bouare, J. Gaudart, P. Knight, R. W. Sauerwein, W. Takken, T. Bousema and O. K. Doumbo (2015).

"Spatial Patterns of Plasmodium falciparum Clinical Incidence, Asymptomatic Parasite Carriage and Anopheles Density in Two Villages in Mali." Am J Trop Med Hyg **93**(4): 790-797.

Smith, D. L., K. E. Battle, S. I. Hay, C. M. Barker, T. W. Scott and F. E. McKenzie (2012). "Ross, macdonald, and a theory for the dynamics and control of mosquito-transmitted pathogens." PLoS Pathog **8**(4): e1002588.

Smith, D. L., C. J. Drakeley, C. Chiyaka and S. I. Hay (2010). "A quantitative analysis of transmission efficiency versus intensity for malaria." Nat Commun **1**: 108.

Smith, D. L., J. Dushoff, R. W. Snow and S. I. Hay (2005). "The entomological inoculation rate and Plasmodium falciparum infection in African children." Nature **438**(7067): 492-495.

Smith, D. L., C. A. Guerra, R. W. Snow and S. I. Hay (2007a). "Standardizing estimates of the Plasmodium falciparum parasite rate." Malar J **6**: 131.

Smith, D. L., F. E. McKenzie, R. W. Snow and S. I. Hay (2007b). "Revisiting the basic reproductive number for malaria and its implications for malaria control." PLoS Biol **5**(3): e42.

Smith, T., G. Killeen, C. Lengeler and M. Tanner (2004). "Relationships between the outcome of Plasmodium falciparum infection and the intensity of transmission in Africa." Am J Trop Med Hyg **71**(2 Suppl): 80-86.

Smith, T., N. Maire, K. Dietz, G. F. Killeen, P. Vounatsou, L. Molineaux and M. Tanner (2006). "Relationship between the entomologic inoculation rate and the force of infection for Plasmodium falciparum malaria." Am J Trop Med Hyg **75**(2 Suppl): 11-18.

Snow, R. W., I. Bastos de Azevedo, B. S. Lowe, E. W. Kabiru, C. G. Nevill, S. Mwankusye, G. Kassiga, K. Marsh and T. Teuscher (1994). "Severe childhood malaria in two areas of markedly different falciparum transmission in east Africa." Acta Trop **57**(4): 289-300.

Snow, R. W., M. H. Craig, U. Deichmann and D. le Sueur (1999). "A preliminary continental risk map for malaria mortality among African children." Parasitol Today **15**(3): 99-104.

Snow, R. W., E. Kibuchi, S. W. Karuri, G. Sang, C. W. Gitonga, C. Mwandawiro, P. Bejon and A. M. Noor (2015). "Changing Malaria Prevalence on the Kenyan Coast since 1974: Climate, Drugs and Vector Control." PLoS One **10**(6): e0128792.

Snow, R. W., C. Molyneux, P. Warn, J. Omumbo, C. Nevill, S. Gupta and K. Marsh (1996). "Infant parasite rates and immunoglobulin M seroprevalence as a measure of exposure to Plasmodium falciparum during a randomized controlled trial of insecticide-treated bed nets on the Kenyan coast." Am J Trop Med Hyg **55**(2): 144-149.

Snow, R. W., J. A. Omumbo, B. Lowe, C. S. Molyneux, J. O. Obiero, A. Palmer, M. W. Weber, M. Pinder, B. Nahlen, C. Obonyo, C. Newbold, S. Gupta and K. Marsh (1997). "Relation between severe malaria morbidity in children and level of Plasmodium falciparum transmission in Africa." Lancet **349**(9066): 1650-1654.

Soares Magalhaes, R. J., N. K. Biritwum, J. O. Gyapong, S. Brooker, Y. Zhang, L. Blair, A. Fenwick and A. C. Clements (2011). "Mapping helminth co-infection and co-intensity: geostatistical prediction in ghana." PLoS Negl Trop Dis **5**(6): e1200.

Soleman, N., D. Chandramohan and K. Shibuya (2006). "Verbal autopsy: current practices and challenges." Bull World Health Organ **84**(3): 239-245.

Soper, F. L. and D. B. Wilson (1943). "Anopheles gambiae in Brazil: 1930 to 1940." New York: Rockefeller Foundation.

Stefani, A., I. Dusfour, A. P. Correa, M. C. Cruz, N. Dessay, A. K. Galardo, C. D. Galardo, R. Girod, M. S. Gomes, H. Gurgel, A. C. Lima, E. S. Moreno, L. Musset, M. Nacher, A. C. Soares, B. Carme and E. Roux (2013). "Land cover, land use and malaria in the Amazon: a systematic literature review of studies using remotely sensed data." Malar J **12**: 192.

Steichen, T. (2001). "METANINF: Stata module to evaluate influence of a single study in meta-analysis estimation." Statistical Software Components.

Sterne, J. A., M. Egger and G. D. Smith (2001). "Systematic reviews in health care: Investigating and dealing with publication and other biases in meta-analysis." Bmj **323**(7304): 101-105.

Sterne, J. A., I. R. White, J. B. Carlin, M. Spratt, P. Royston, M. G. Kenward, A. M. Wood and J. R. Carpenter (2009). "Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls." Bmj **338**: b2393.

Stewart, L., R. Gosling, J. Griffin, S. Gesase, J. Campo, R. Hashim, P. Masika, J. Mosha, T. Bousema, S. Shekalaghe, J. Cook, P. Corran, A. Ghani, E. M. Riley and C. Drakeley (2009). "Rapid assessment of malaria transmission using age-specific sero-conversion rates." PLoS One **4**(6): e6083.

Stresman, G. H., E. Giorgi, A. Baidjoe, P. Knight, W. Odongo, C. Owaga, S. Shagari, E. Makori, J. Stevenson, C. Drakeley, J. Cox, T. Bousema and P. J. Diggle (2017). "Impact of metric and sample size on determining malaria hotspot boundaries." Sci Rep **7**: 45849.

Stresman, G. H., A. Kamanga, P. Moono, H. Hamapumbu, S. Mharakurwa, T. Kobayashi, W. J. Moss and C. Shiff (2010). "A method of active case detection to target reservoirs of asymptomatic malaria and gametocyte carriers in a rural area in Southern Province, Zambia." Malar J **9**: 265.

Strode, C., S. Donegan, P. Garner, A. A. Enayati and J. Hemingway (2014). "The impact of pyrethroid resistance on the efficacy of insecticide-treated bed nets against African anopheline mosquitoes: systematic review and meta-analysis." PLoS Med **11**(3): e1001619.

Sturrock, H. J., M. S. Hsiang, J. M. Cohen, D. L. Smith, B. Greenhouse, T. Bousema and R. D. Gosling (2013). "Targeting asymptomatic malaria infections: active surveillance in control and elimination." PLoS Med **10**(6): e1001467.

Sutherland, C. J., N. Tanomsing, D. Nolder, M. Oguike, C. Jennison, S. Pukrittayakamee, C. Dolecek, T. T. Hien, V. E. do Rosario, A. P. Arez, J. Pinto, P. Michon, A. A. Escalante, F. Nosten, M. Burke, R. Lee, M. Blaze, T. D. Otto, J. W. Barnwell, A. Pain, J. Williams, N. J. White, N. P. Day,

G. Snounou, P. J. Lockhart, P. L. Chiodini, M. Imwong and S. D. Polley (2010). "Two nonrecombining sympatric forms of the human malaria parasite Plasmodium ovale occur globally." J Infect Dis **201**(10): 1544-1550.

Tatem, A. J., S. J. Goetz and S. I. Hay (2004). "Terra and Aqua: new data for epidemiology and public health." Int J Appl Earth Obs Geoinf **6**(1): 33-46.

Team, R. C. (2016). "R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria." R core.

Tiono, A. B., A. Ouedraogo, B. Ogutu, A. Diarra, S. Coulibaly, A. Gansane, S. B. Sirima, G. O'Neil, A. Mukhopadhyay and K. Hamed (2013). "A controlled, parallel, cluster-randomized trial of community-wide screening and treatment of asymptomatic carriers of Plasmodium falciparum in Burkina Faso." Malar J **12**: 79.

Tobler, W. R. (1970). "A Computer Movie Simulating Urban Growth in the Detroit Region." Economic Geography **46**: 234-240.

Trape, J. F., M. C. Quinet, S. Nzingoula, P. Senga, F. Tchichelle, B. Carme, D. Candito, H. Mayanda and A. Zoulani (1987). "Malaria and urbanization in central Africa: the example of Brazzaville. Part V: Pernicious attacks and mortality." Trans R Soc Trop Med Hyg **81 Suppl 2**: 34-42.

Trape, J. F., A. Tall, N. Diagne, O. Ndiath, A. B. Ly, J. Faye, F. Dieye-Ba, C. Roucher, C. Bouganali, A. Badiane, F. D. Sarr, C. Mazenot, A. Toure-Balde, D. Raoult, P. Druilhe, O. Mercereau-Puijalon, C. Rogier and C. Sokhna (2011). "Malaria morbidity and pyrethroid resistance after the introduction of insecticide-treated bednets and artemisinin-based combination therapies: a longitudinal study." Lancet Infect Dis **11**(12): 925-932.

Tusting, L. S., C. Bottomley, H. Gibson, I. Kleinschmidt, A. J. Tatem, S. W. Lindsay and P. W. Gething (2017). "Housing Improvements and Malaria Risk in Sub-Saharan Africa: A Multi-Country Analysis of Survey Data." PLoS Med **14**(2): e1002234.

Tusting, L. S., T. Bousema, D. L. Smith and C. Drakeley (2014). "Measuring changes in Plasmodium falciparum transmission: precision, accuracy and costs of metrics." Adv Parasitol **84**: 151-208.

Tusting, L. S., M. M. Ippolito, B. A. Willey, I. Kleinschmidt, G. Dorsey, R. D. Gosling and S. W. Lindsay (2015). "The evidence for improving housing to reduce malaria: a systematic review and meta-analysis." Malar J **14**: 209.

Tusting, L. S., B. Willey, H. Lucas, J. Thompson, H. T. Kafy, R. Smith and S. W. Lindsay (2013). "Socioeconomic development as an intervention against malaria: a systematic review and meta-analysis." Lancet **382**(9896): 963-972.

Viana, M., A. Hughes, J. Matthiopoulos, H. Ranson and H. M. Ferguson (2016). "Delayed mortality effects cut the malaria transmission potential of insecticide-resistant mosquitoes." Proc Natl Acad Sci U S A **113**(32): 8975-8980.

von Seidlein, L. and B. M. Greenwood (2003). "Mass administrations of antimalarial drugs." Trends Parasitol **19**(10): 452-460.

von Seidlein, L., K. Ikonomidis, R. Bruun, M. Jawara, M. Pinder, B. G. Knols and J. B. Knudsen (2012a). "Airflow attenuation and bed net utilization: observations from Africa and Asia." Malaria journal **11**(1): 200.

von Seidlein, L. and J. Knudsen (2016). "Malaria Epidemiology in Kilifi, Kenya during the 21st Century: What Next?" PLoS Med **13**(6): e1002048.

von Seidlein, L., R. Olaosebikan, I. C. Hendriksen, S. J. Lee, O. T. Adedoyin, T. Agbenyega, S. B. Nguah, K. Bojang, J. L. Deen, J. Evans, C. I. Fanello, E. Gomes, A. J. Pedro, C. Kahabuka, C. Karema, E. Kivaya, K. Maitland, O. A. Mokuolu, G. Mtove, J. Mwanga-Amumpaire, B. Nadjm, M. Nansumba, W. P. Ngum, M. A. Onyamboko, H. Reyburn, T. Sakulthaew, K. Silamut, A. K. Tshefu, N. Umulisa, S. Gesase, N. P. Day, N. J. White and A. M. Dondorp (2012b). "Predicting

the clinical outcome of severe falciparum malaria in african children: findings from a large randomized trial." Clin Infect Dis **54**(8): 1080-1090.

Wanjala, C. L., J. Waitumbi, G. Zhou and A. K. Githeko (2011). "Identification of malaria transmission and epidemic hotspots in the western Kenya highlands: its application to malaria epidemic prediction." Parasit Vectors **4**: 81.

Weiss, D. J., S. Bhatt, B. Mappin, T. P. Van Boeckel, D. L. Smith, S. I. Hay and P. W. Gething (2014). "Air temperature suitability for Plasmodium falciparum malaria transmission in Africa 2000-2012: a high-resolution spatiotemporal prediction." Malar J **13**(1): 171.

Wheeler, D. C. (2007). "A comparison of spatial clustering and cluster detection techniques for childhood leukemia incidence in Ohio, 1996–2003." International Journal of Health Geographics **6**(1): 13.

White, I. R., P. Royston and A. M. Wood (2011). "Multiple imputation using chained equations: issues and guidance for practice." Statistics in medicine **30**(4): 377-399.

White, N. J. (2008). "Plasmodium knowlesi: The Fifth Human Malaria Parasite." Clinical Infectious Diseases **46**(2): 172-173.

WHO (2006a). "Indoor residual spraying: use of indoor residual spraying for scaling up global malaria control and elimination: WHO position statement."

WHO (2006b). "Malaria vaccine technology roadmap."

WHO (2007). Malaria elimination: a field manual for low and moderate endemic countries.

WHO (2010). "World Malaria Report " World Health Organization.

WHO (2012a). "Global Plan for Insecticide Resistance Management in Malaria Vectors (GPIRM)." WHO: Geneva, Switzerland.

WHO (2012b). "Malaria Rapid Diagnostic Test Performance. Result of WHO Product Testing of Malaria RDT: Round 4." WHO.

WHO (2012c). World malaria report 2012 Fact Sheet.

WHO (2013a). "Malaria Diagnosis in low Transmission Settings (Meeting Report)." WHO.

WHO (2013b). "Malaria entomology and vector control. Guide for participants." Geneva: WHO.

WHO (2013c). "WHO policy brief for the implementation of intermittent preventive treatment of malaria in pregnancy using sulfadoxine-pyrimethamine (IPTp-SP)." WHO.

WHO (2015a). "Guidelines for treatment of malaria (Third edition)." WHO.

WHO (2015b). "World Malaria Report 2015." Geneva: World Health Organization.

WHO (2016). "Status report: Artemisinin and artemisinin-based combination therapy resistance. ." Geneva: WHO.

WHO (2017). "Ghana, Kenya and Malawi to take part in WHO malaria vaccine pilot programme." Geneva, WHO.

Willey, B. A., J. R. Armstrong Schellenberg, W. Maokola, K. Shirima, M. Chemba, H. Mshinda, P. Alonso, M. Tanner and D. Schellenberg (2011). "Evaluating the effectiveness of IPTi on malaria using routine health information from sentinel health centres in southern Tanzania." Malar J **10**(1): 41.

Wilson, A. L. (2011). "A systematic review and meta-analysis of the efficacy and safety of intermittent preventive treatment of malaria in children (IPTc)." PloS one **6**(2): e16976.

Wimberly, M. C., P. Giacomo, L. Kightlinger and M. B. Hildreth (2013). "Spatio-temporal epidemiology of human West Nile virus disease in South Dakota." Int J Environ Res Public Health **10**(11): 5584-5602.

Wirth, D. F. (2002). "Biological revelations." Nature **419**(6906): 495-496.

Woolhouse, M. E. (1998). "Patterns in parasite epidemiology: the peak shift." Parasitol Today **14**(10): 428-434.

Woolhouse, M. E., C. Dye, J. F. Etard, T. Smith, J. D. Charlwood and G. P. Garnett (1997a). "Heterogeneities in the transmission of infectious agents: implications for the design of control programs." Proc Natl Acad Sci U S A **94**.

Woolhouse, M. E., C. Dye, J. F. Etard, T. Smith, J. D. Charlwood, G. P. Garnett, P. Hagan, J. L. Hii, P. D. Ndhlovu, R. J. Quinnell, C. H. Watts, S. K. Chandiwana and R. M. Anderson (1997b). "Heterogeneities in the transmission of infectious agents: implications for the design of control programs." Proc Natl Acad Sci U S A **94**(1): 338-342.

Wu, L., L. L. van den Hoogen, H. Slater, P. G. Walker, A. C. Ghani, C. J. Drakeley and L. C. Okell (2015). "Comparison of diagnostics for the detection of asymptomatic Plasmodium falciparum infections to inform control and elimination strategies." Nature **528**(7580): S86-93.

Yeboah-Antwi, K., J. O. Gyapong, I. K. Asare, G. Barnish, D. B. Evans and S. Adjei (2001). "Impact of prepackaging antimalarial drugs on cost to patients and compliance with treatment." Bull World Health Organ **79**(5): 394-399.

Yeshiwondim, A. K., S. Gopal, A. T. Hailemariam, D. O. Dengela and H. P. Patel (2009). "Spatial analysis of malaria incidence at the village level in areas with unstable transmission in Ethiopia." Int J Health Geogr **8**: 5.

Zegers de Beyl, C., H. Koenker, A. Acosta, E. O. Onyefunafoa, E. Adegbe, A. McCartney-Melstad, R. A. Selby and A. Kilian (2016). "Multi-country comparison of delivery strategies for mass campaigns to achieve universal coverage with insecticide-treated nets: what works best?" Malar J **15**: 58.